Ecological Aspects of Vegetation Establishment on Landfills

by

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ABSTRACT

A high level of plant mortality on the Bisasar Road landfill, Durban, South Africa initiated an investigation into the primary causes of the mortalities and a search for potentially tolerant plant species. Field studies revealed that volunteer grass growth on cover soils was primarily limited by elevated soil CO_2, with high soil conductivity and low soil moisture possibly compounding the effect. *Cynodon dactylon*, the most abundant coloniser of the site appeared to be relatively sensitive to high soil CO_2, whilst less common species such as *Sporobolis africanus* and *Paspalum paspoloides* appeared to be less sensitive.

Further research focused on the high mortality of trees planted on the landfill providing insight into the important variables limiting survival and the relative differences in performance of 20 tree species. A more rigorous 14-month field experiment was designed and constructed, to assess the performance of 10 of the more promising tree species, the environmental conditions limiting tree growth and the benefit of a deeper layer of better quality topsoil. Some species, such as *Barringtonia racemosa*, performed relatively well in the field experiment, whilst other species such as *Syzygium cordatum*, and *Harpephyllum caffrum* experienced high mortalities and poor growth. The better quality topsoil layer provided little improvement in the performance of the stronger or the weaker species, however significant improvements were recorded for species with relatively intermediate performance. The composition of the soil atmosphere was shown to determine rooting depth. Species that performed better had deeper roots, possibly assisting them in utilising deeper soil moisture reserves. It was concluded that high soil CO_2 and low soil O_2 levels were the key variables responsible for poor tree survival and growth in this field experiment.

A soil fumigation system was designed to provide more control of soil gas concentrations and to experimentally investigate differential species responses and the relative effects of soil CO_2 and O_2 on tree survival and growth. The apparatus fumigated, for a period of 140 days, the rhizosphere of 80 potted 'tolerant' (*Barringtonia racemosa*) and 'non tolerant' (*Harpephyllum caffrum*) trees with 4 treatments consisting of varying combinations of CO_2 and O_2. The difference in performance of *Barringtonia racemosa* and *Harpephyllum caffrum* in the experiment on the landfill was similar to that of the elevated CO_2 low O_2 fumigation treatment, supporting the premise that landfill gas was the key cause for poor performance of plants. Reduced stomatal conductance and resultant limitations on photosynthesis were found to be indicative of species sensitivity. Low O_2 had an additive effect on the impact of elevated CO_2 in *Harpephyllum caffrum* however, even with normal soil O_2 levels, 25% soil CO_2 had negative growth effects on this sensitive species. Maintenance of plant health and better performance of *Barringtonia* was attributed to a high inherent level of tissue porosity and aerenchyma. The research provided a greater understanding of the causes of poor vegetation growth and the possible mechanisms of species tolerance to landfill conditions.

PREFACE

The experimental work described in this thesis was carried out in the School of Life and Environmental Sciences, University of Natal, Durban, from January 1996 to December 2002, under the supervision of Professor J.A. Cooke.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 SOLID WASTE AND LANDFILLS IN SOUTH AFRICA

Waste can be generated from residential, commercial or industrial activity and can consist of unwanted by–products or the remainder of any process, be it a gas, liquid, solid or a combination. It is estimated that South Africa produces between 340 and 480 million tonnes of solid waste annually (CSIR, 1991 from WRC, 1995). More recent estimates of waste volumes suggest that approximately 42 million m^3 of general waste is produced per year across the country (Burger, 2001) The major sources of solid waste are shown in Table 1.1.

Table 1.1: Sources of waste generation in South Africa

Source of waste	Annual Production ($x10^6$ tonnes)
Mining	238.5
Fly Ash	22.2
Agriculture	20.0
Municipal Waste	15.0
Chemical Waste	12.2
Sewage Sludge	12.0
Metallurgical Waste	5.4
Unclassified	4.8

CSIR, 1991 from WRC, 1995

A well-managed sanitary landfill provides an economically and safe means of waste disposal. A sanitary landfill site is a carefully selected, designed and managed waste disposal and containment operation. The waste delivered on a daily basis to the site is spread compacted and covered with soil according to a pre-planned site development programme. Waste deposited in landfills can be broadly classified as general or hazardous waste. General waste includes rubble, garden refuse, domestic waste, commercial waste and general dry industrial waste. Hazardous waste includes any matter, which has toxic

chemicals or long lasting properties, which may have a harmful effect on human health or the environment. The level of site regulation and control determines what types of waste are acceptable for disposal at a site. General waste is disposed of in G classified sites, low hazardous material in H:h classified sites, whilst highly hazardous material can only be disposed in H:H classified landfills which have the highest level of regulation and control.

Of the total waste produced in South Africa an estimated 12 million tonnes is disposed of in sanitary landfills (Jarmain *et al*, 1994) of which South Africa currently has 638 operational sites, 49 officially closed sites and 43 proposed new landfills (Burger, 2001). The odour, noise, dust and visual impact of these operational landfill sites can disturb surrounding communities and trigger more serious concerns about impacts on community health and property value. The use of vegetation with careful landscaping and the construction of berms (an artificial ridge or embankment) can stabilise the completed sections of the site, reduce dust, absorb noise and improve the visual impact of the site (Zeiss & Atwater, 1993). Thus, the successful establishment of trees and grasses on operational landfills can make a vital contribution towards reducing the impact of the site. Successful establishment of vegetation is also essential on completed landfills. Due to the production of flammable gas and site settling as the waste degrades, rehabilitation of closed landfills is usually limited to parks, sports fields and other similar amenity after-uses (Aplet & Conn, 1977; Cooper *et al* 1997; Gilman *et al* 1982; Robinson & Handel, 1995), all of which require successful vegetation establishment. However, the revegetation of landfills throughout the world has met with many difficulties due to the harsh environmental conditions commonly found on landfills (Chan *et al* 1997; Chan *et al* 1996; Chan *et al* 1991; Ettala *et al* 1988; Gilman *et al* 1982; Lassini *et al* 1997; Leone *et al* 1983; Moffat & Houston, 1991; Wong & Yu, 1989; Wong, 1988).

1.2 LANDFILLS AND REHABILITATION

In South Africa the Department of Water Affairs and Forestry, state in section 12, Minimum Requirements for Waste Disposal by Landfill (2nd ed. 1998), that the final condition of the site must be environmentally acceptable and there will be no long-term effects on the surrounding area, water regime and population. It also stipulates that vegetation planted for the purposes of rehabilitation, erosion control or beautification must be maintained to ensure it achieves its purpose. There are no further specifications or guidance given as to revegetation. The regulations do however stipulate the need for incorporation of a 'low permeability' layer or cap in the final cover system of landfills, this is a common requirement throughout the world (Fourie, 2002). The 'low permeability' layer reduces rainfall ingress into the waste, which results in less leachate production, and helps control landfill gas escape into the atmosphere. In South Africa the final cover requirements can vary as considerations of regional climatic and site specific conditions are made. However, a typical cover requirement for a large municipal waste disposal site consists of a 300mm compacted clayey 'low permeability' layer covered with a relatively thin 200mm topsoil layer (Fourie, 2002).

Although there are no guidelines in South Africa stipulating what should be planted on landfills during rehabilitation, grass is the most common forming sport fields or open grassland. However, recently in South Africa there is a demand for rehabilitation to recognise the ecological diversity of a functional ecosystem and assist in the conservation of indigenous fauna and flora (Strachan *et al* 2002). This promotes the use of a broader variety of plant species and the incorporation of shrubs and trees in the rehabilitation plan.

There have been reservations, internationally, about the use of trees in landfill revegetation. Concern about damage to the integrity of the landfill cap by trees has been expressed. In particular: the penetration of the landfill cap by tree roots; evapo-transpiration resulting in shrinkage and cracking of clay cap; and trees experiencing windthrow may disrupt the integrity of the landfill cap. These concerns have previously resulted in the recommendation in the United Kingdom and the United States that trees should not be planted on landfills that have a 'low permeability' layer (Dobson & Moffat, 1995).

However, no evidence, direct or indirect, has been found to support these potential problems on which the recommendations were based (Robinson & Handel, 1995). These fears have since been proven to be largely a misconception due to the lack of knowledge regarding tree root growth characteristics. In fact, evidence suggests that trees show no threat to the integrity of a clay or geotextile covering on landfill sites.(Dobson & Moffat, 1994; Crook, 1992; Robinson & Handel, 1995; Simmons & Coulter, 1997). Some plants are known to produce extremely deep root systems, however this is largely dependent on the particular soil environment (Dobson & Moffat, 1994; Ruark *et al* 1982). The bulk densities found to prevent tree root growth are usually much lower than the recommended bulk density of engineered clay caps (Dobson & Moffat, 1994; Robinson & Handel, 1995). Roots also tend to avoid inhospitable soil zones such as that created by the underlying waste. These findings have resulted in the latest government guidance in the United Kingdom recommending that trees may be planted on all types of landfills (ODP, 2000; Simmons, 1999).

Research indicates that trees can be planted on capped and uncapped sites without compromising the effectiveness of pollution control systems. This allows for a more varied landscape design on all types of landfills and enables sites to blend better with the surroundings and increases the scope of after-uses (Simmons & Coulter, 1997). However, the usually shallow topsoil depth on landfills and the potential for windthrow of older and taller trees has lead to the recommendation that once trees have reached a certain height a system of coppicing should be implemented so as to maintain their stability (Ballardini & Lassini, 1997).

In the South African context the knowledge that trees can safely be used on capped landfills allows landfill rehabilitation plans to incorporate more complex after-use goals without concern for the integrity of the 'low permeability' layer. However, currently there also questions being raised about the necessity for 'low permeability' layer in the rehabilitation plan. Compacted clay capping systems tend to work well in temperate climates that have an excess of precipitation over evaporation because the clay layer does not dry and maintains its flexibility. However, such systems do not perform as well in semi-arid environments, which most of South Africa is classified, as the clay cap dries and cracks becoming permeable. Therefore, it has been suggested that a cover that stores moisture during particularly wet periods and releases moisture via evaporation and evapo-transpiration during subsequent dry periods is a better option. Such an alternative landfill cover would consist, in its simplest form, of a single layer of silty or sandy soil with negligible amounts of clay. The soil used in this cover would resist linear shrinkage thus maintaining flexibility and not cracking during dry periods. The cover would not be considered a barrier but more a regulator of landfill emissions, as it would control moisture into the landfill and would be designed to promote methane oxidation, thus reducing

landfill gas emission into the atmosphere. Although this explanation of an alternative landfill cover is over simplified, a more detailed explanation and evidence supporting this idea as a viable concept are provided by Fourie, (2002). However, if this concept were implemented the greater level of interaction between underlying wastes and the surface soils used for revegetation would be a major consideration.

Similarly, the cover soils used in operational landfills that require stabilisation and aesthetic improvement using vegetation often do not have any 'low permeability' layer separating the topsoil from the underlying waste. Therefore, there is a demand for knowledge about the interactions between the waste and the soil layers used for plant growth and how this can influence successful vegetation establishment. Furthermore, with the operational life span of landfills often exceeding 30 years the demand for plants that can grow successfully on operational sites is ever increasing.

1.3 PLANTS AND THE LANDFILL ENVIRONMENT

The establishment of vegetation on landfills, especially older sites, which have lower standards of pollution control and restoration, frequently results in high plant mortality and sometimes in complete failure. The unsuccessful establishment of vegetation on landfills has been attributed to many factors which include the following: landfill gas; toxic leachate; elevated soil temperature; shallow soil; poor soil quality; poor soil structure; waterlogging; drought; damage by animals; air pollution; and vandalism (Barry, 1987; Dobson & Moffat, 1994; Graber, 1999).

When refuse is first deposited into a new landfill it still contains oxygen, which results in aerobic decomposition. This primarily produces carbon dioxide and water. Within six months all oxygen within the waste is usually used up and decomposition continues in an anaerobic manner (Flower, *et al* 1981). With very little oxygen within the soil, facultative and obligate anaerobic bacterial populations proliferate. These organisms break down or use various organic and inorganic compounds so as to provide their metabolic energy. However, instead of using oxygen as an electron acceptor they utilise inorganic (anaerobic respiration) or organic (fermentation) substrates as the terminal electron acceptors (Bogner, 1992; Gambrell and Patrick, 1978). The result is a different end products of organic decomposition, by comparison to aerobic environments, such as methane, hydrogen, ammonia, amines, mercaptans, butyric acid and hydrogen sulphide, many of which can lead to poor plant growth and survival (Dobson & Moffat 1994; Gambrell and Patrick, 1978; Leone *et al* 1977). Decomposition usually remains in the anaerobic phase because waste compaction and soil cover limit oxygen diffusion to the immediate surface layers (Flower, *et al* 1981).

A typical landfill gas composition consists of 64% methane (CH_4), 34% carbon dioxide (CO_2) and trace concentrations of a wide range of organic gases. These gases escape through the landfill substrates along the paths of least resistance (Christophersen *et al* 2001; Dobson & Moffat, 1994; Flower, *et al* 1981). The composition by volume of a typical landfill gas is given in Table 1.2.

Table 1.2: Typical landfill gas composition

Component	Typical value (% volume)	Max. value measured (% volume)
Methane	63.8	88.0
Carbon dioxide	33.6	89.0
Oxygen	0.16	20.9
Nitrogen	2.4	87.0
Hydrogen	0.05	21.1
Carbon monoxide	0.001	0.0
Ethane	0.005	0.0139
Ethene (ethylene)	0.018	-
Acetaldehyde	0.005	-
Propane	0.002	0.0171
Butane	0.003	0.023
Helium	0.00005	-
Higher alkanes	<0.05	0.07
Unsaturated hydrocarbons	0.009	0.048
Halogenated hydrocarbons	0.00002	0.032
Hydrogen sulphide	0.00002	35.0
Organosulphur compounds	0.00001	0.028
Alcohols	0.00001	0.127
Others	0.00005	0.023

From Waste Management Paper No. 27 (DoE, 1991b) from Dobson & Moffat, 1994

A survey of 65 sanitary landfill sites in the United States revealed that among all the reported environmental factors potentially limiting vegetation establishment on landfills, high levels of landfill gas in the soil was the main cause (Leone and Flower, 1982). This is not a unique finding and a correlation between the level of landfill gas in the soil and poor plant performance has also been noted in numerous other studies (Bradshaw & Chadwick, 1980; Chan *et al*, 1991; Flower *et al*, 1981; Flower *et al*, 1977; Spreul & Cullum, 1987). The harmful effects of landfill gas are usually attributed to displacement of oxygen and resultant anaerobic conditions within the root zone and not the toxic effects of methane or trace components of landfill gas (Barry, 1987; Cole, *et al* 1978; Dobson & Moffat, 1994; Flower *et al*, 1981). However, carbon dioxide (CO_2) which makes up a large component

of landfill gas is also widely accepted to be a problem for plant growth and survival when above certain concentrations (Arthur, *et al* 1981; Barry, 1987; Chan *et al,* 1991; Dobson & Moffat, 1994; Flower *et al,* 1981). There is not an extensive literature on the impact of elevated soil CO_2 on plants however, some of the available ideas will be reviewed below and then the more extensive knowledge base on plants and low soil O_2 will be discussed.

Most soils contain methanotrophic bacteria capable of oxidising methane in the presence of oxygen into CO_2 and water as given in the following equation:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

Thus, not only is CO_2 a large component of the original landfill gas, a large portion of the methane component can also be converted into CO_2 within landfill cover soils. This can result in further increases in CO_2 levels and further depletion of soil O_2 (De Rome *et al,* 1997; Dobson & Moffat, 1994; Hoeks, 1983). Due to methane oxidation the concentration of CO_2 in landfill gas tends to increase as the mixture of gases gets closer to the soil surface and more oxygen is available (Haarstad, 1997). However, the depth of oxidation can vary with soil structure and is greatest where the diffusion of oxygen from the atmosphere and methane overlap (Kightley *et al* 1995). This is usually within the top 300mm of soil, thus resulting in the depletion of soil O_2 and an increase in CO_2 in the root zone for most plants (Dobson & Moffat, 1994). The methanotrophic bacteria responsible for methane oxidation can also produce intermediate products such as methanol, formaldehyde and formic acid (De Rome *et al* 1997; Brown *et al* 1964). These

intermediate products and high levels of carbon dioxide may exhibit direct toxicity to plants.

The CO_2 concentrations within the soil gas phase of normal soils is between 1-5% (Geisler, 1963; Gendebien *et al* 1992; Santruckova & Simek, 1997). However, under landfill conditions root zone CO_2 levels are commonly found in excess of 15% by volume of the soil atmosphere (Chan *et al* 1991; Gilman *et al* 1982; Leone *et al* 1977; Wong *et al* 1992). Concentrations of CO_2 as high as 43% have been measured at a 30cm depth on landfill sites and concentrations as high as 75% by volume could theoretically occur (Arthur *et al* 1981). A large variation in species tolerance to CO_2 levels has been reported (Arthur, *et al* 1981, Gendebien *et al*, 1992; Leone *et al* 1977), and the lower the oxygen levels in conjunction with high CO_2 levels, the greater the degree of toxicity (Ruark *et al* 1982). Generally CO_2 concentrations in excess 6.5% in the rhizosphere has been found to inhibit root growth and result in poor health of number of plant species (Conlin & Van den Driessche, 2000; Chang and Loomis, 1945; Nobel, 1990). Thus, soil CO_2 levels in landfill soils could have a marked effect on plant growth and survival. The mechanism by which elevated CO_2 in the rhizosphere effects plants is not entirely clear. However, as for other components of landfill gas, CO_2 contributes to the displacement of oxygen and the development of anaerobic soil conditions (Flower *et al* 1981, Gendebien *et al*, 1992). It has also been suggested that the effects of high soil CO_2 may be related to the formation of carbonic acid and the acidification of soil water caused by the dissolution of CO_2 which is highly soluble in water (Santruckova & Simek, 1997). The resultant lowering of soil solution pH and possibly changes to the internal pH of cells has been suggested as one of

the possible factors contributing to CO_2 toxicity (Flower *et al* 1981; Santruckova & Simek, 1997).

There has been much study on the mechanisms by which low soil O_2 conditions cause plant stress. A number of key possible causes of root cell damage have been identified and these include insufficient energy generation to sustain cell integrity; cell poisoning by ethanol formed by alcoholic fermentation and cytoplasmic acidosis caused by the products of anaerobic respiration (Vartapetian and Jackson, 1997). It is important to note that when soil O_2 levels are low, anaerobic respiration is likely to occur resulting in a sharp decline in energy availability (Mathews and van Holde, 1991). The reduction in available energy can also reduce the active uptake of mineral nutrients (Kozlowski, 1986; Veen, 1987). Therefore, low oxygen conditions in the soil can result in potassium, nitrogen, phosphorus, calcium and magnesium deficiencies in plants (Flower *et al* 1981, Leone *et al* 1977, Taiz & Zeiger, 1998).

There are also a number of indirect effects by which low soil O_2 conditions can effect plant survival and growth. Under anaerobic soil conditions the release of organic acids by micro-organisms and the accumulation of carbonic acid from respiration and fermentation often results in soil acidification (Flower *et al*, 1981, Larcher, 1980). Low soil pH and redox potentials often accompany the anaerobic conditions. The resultant reducing conditions can lead to increased metal solubility, such as for iron, manganese, aluminium, copper and zinc. The reduced metals can become more available to plants in higher (toxic) concentrations (Crawford, 1989; Leone & Flower 1982). Interestingly, this increased

availability of metals has the potential to result in phytotoxicty however no effects on landfills other than enhancing the trace metal nutrient status of cover soils has been reported (Leone & Flower, 1982).

Microbial activity under low soil O_2 conditions can also change some of the characteristics of the soil, particularly reducing the organic carbon to nitrogen ratio (Flower *et al*, 1981). Nitrogen deficiencies limiting plant growth are common in anaerobic systems because physical, chemical and biological processes under these conditions favour denitrification and low nitrate assimilation. Denitrification is the reduction of nitrate and /or nitrite nitrogen to volatile gases, mainly nitrous oxide and molecular nitrogen, that may escape into the atmosphere (Gambrell and Patrick, 1978).

The poor air movement in the soil atmosphere, commonly found in anaerobic soils, may cause ethylene, a natural plant hormone, produced by the plant, to accumulate in the root tissue and surrounding soil (Jackson, 1985). However, the decomposition of waste in landfills under low oxygen conditions also produces ethylene that can infiltrate the root zone of plants on landfills (Zacharias, 1995). High levels of ethylene can inhibit plant growth (Pezeshki, *et al* 1993; Seliskar, 1988), cause leaf chlorosis (Gepstein and Thimann, 1981; Jackson, *et al* 1987) and cause plant death (Jackson, 1985). Ethylene typically occurs in concentrations of 180ppm (v/v) in landfill gas (Spruell & Cullum, 1987, Dobson & Moffat, 1994) (Table 1.2). However, it is responsible for a greater than 50% reduction in plant growth and often death at concentrations less than 10ppm (Dobson & Moffat, 1994, Smith and Restall, 1971; Spreull & Cullum, 1987). However, Tosh *et al* (1994) found the

threshold concentration for silver birch (*Betula pendula*) seedlings to be 80 ppm, suggesting that there maybe considerable variation in species tolerance. Nevertheless, ethylene may be an important component of landfill gas in determining plant response and vegetation establishment on landfills.

The concentrations of landfill gases in the root zone can be reduced by active extraction or passive venting of gases, from the decomposing waste, which can be burnt off as a flare or used as a fuel (Flower, *et al* 1981; Leone, *et al* 1977). Another alternative is the establishment of gas barriers, using a compacted clay layer or geotextile, preventing landfill gas infiltrating the root zone (Flower *et al* 1981). These procedures can alleviate the problems associated with resultant poor carbon dioxide, oxygen and possibly ethylene levels in the soil. However, they are expensive and not an option for operational sites where revegetation maybe temporary. It is also difficult to install gas extraction or barrier systems in old closed landfill sites that were designed before landfill gas control measures were considered a necessity. Therefore the use of plant species tolerant to the effects of landfill gas infiltration into the soil are the best option for attaining successful revegetation.

In terms of finding species with potential tolerance to these effects, it has been noted that there are many similarities between the anaerobic conditions caused by landfill gas and that of soil waterlogging (Barry, 1987, Chan *et al*, 1991). This has commonly lead to the proposal that species adapted to soil flooding are potentially suitable for planting on landfills (Arthur *et al* 1981; Gilman *et al* 1985; Leone *et al* 1977; Zhang *et al* 1995). The most widespread anatomical feature conferring tolerance to flooded soils is an

interconnected system of gas spaces (aerenchyma) within the root and stem of plants (Jackson, 1994). Aerenchyma results in a lower number of energy-demanding cortical cells in the roots, thus lowering the demand for oxygen (Drew & Fourcy, 1986; Drew & Saker, 1986). It also enhances internal oxygen diffusion and allows oxygen transport from the shoots to the roots (Jackson & Attwood, 1996; Kludze *et al* 1994; Wiedenroth, 1993). Aerenchyma tissue can also result in the oxidation of the rhizosphere, thus alleviating some of the problems associated with low redox potentials caused by anaerobic soils, such as metal toxicity (Blom, 1999; Crawford, 1989). Therefore, species with characteristics conferring tolerance to flooded soils may have attributes that would be beneficial for growth and survival in soils infiltrated with landfill gas.

Apart from landfill gas other interactions between the underlying waste and cover soils, such as heat transfer and leachate contamination can cause changes to the soil that could limit plant survival and growth. The temperature of landfill soils is frequently higher than that of native soils because anaerobic decomposition of waste is exothermic (Flower *et al* 1981, Gilman *et al* 1981, Maurice & Lagerkvist, 1997). High soil temperatures are usually associated with high landfill gas emissions, as warm landfill gas infiltration into the cover soils is usually the key mode of temperature transfer from the waste (Chan *et al* 1991). Elevated temperatures of between 30-40 °C are often measured within the topsoil of landfill sites (Chan *et al* 1991; Dobson & Moffat, 1994; Moffat & Houston 1991), sometimes the temperature difference can be greater than 30 °C between anaerobic and adjacent aerobic soils (Leone *et al* 1977).

Root growth has been found to decrease significantly within the temperature range of 25-35 °C (Ruark *et al*, 1982). Therefore it is not surprising that elevated soil temperature has been identified as a potential problem for plant growth on landfills (Gilman *et al*, 1982; Moffat & Houston, 1991). Although, the higher soil temperature on landfills can prevent the winter freezing of soil water and extend the growing season of many plants in colder countries (Chan *et al* 1991). In the sub-tropical climate of southern Africa the freezing of soil water is seldom encounted. In such tropical climates the raised soil temperature is likely to present a problem for vegetation growth, especially, if one considers that higher soil temperatures enhance the oxygen demand of the root in a soil low in oxygen (Flower *et al* 1981; Gendebien *et al* 1992). However, the amount of heat transferred from the decomposing waste can be alleviate by greater soil depth, the further plant roots are from the source of heat the closer the soil temperature is to ambient (Moffat & Houston, 1991).

An assessment of the impacts of soil leachate contamination on landfill cover soils is not simple. Leachate is the diverse mixture of dissolved and suspended organic and inorganic materials formed when the products of biodegradation mix with the downward migration of water through a landfill (Cooper *et al* 1997). The composition of leachate changes with time as the biodegradation process proceeds, it will also vary with the disposal of wastes of different composition. An example of leachate composition from a recent and an aged domestic waste disposal landfill is given in Table 1.3. With the onset of anaerobiosis as molecular oxygen is depleted the redox potential falls and increases the concentrations of soluble reduced-state metals, such as iron and manganese (Rees, 1982). These metals precipitate as sulphides, hydroxides and carbonates as the pH rises (Rees, 1982). This

results in a considerable reduction in the concentrations of these metals in leachate as the landfill ages (Table 1.3).

Table 1.3: Typical composition of leachates from recent and aged domestic wastes (all figures in mg l^{-1} except pH)

	Leachates from recent wastes (3 years)	Leachate from aged wastes (10 years)
pH	6.2	7.5
COD (Chemical oxygen demand)	23800	1160
BOD (Biochemical oxygen demand)	11900	260
TOC (Total organic carbon)	8000	465
Fatty acids	5688	5
Ammoniacal-N	790	370
Oxidised-N	3	1
p-Phosphate	0.73	1.4
Chloride	1315	2080
Sodium (Na)	960	1300
Magnesium (Mg)	252	185
Potassium (K)	780	590
Calcium (Ca)	1820	250
Manganese (Mn)	27	2.1
Iron (Fe)	540	23
Nickel (Ni)	0.6	0.1
Copper (Cu)	0.12	0.3
Zinc (Zn)	21.5	0.4
Lead (Pb)	8.4	0.14

Adapted from Christensen *et al* 2001 and Fell, *et al* 1993

When leachate is not properly contained it can contaminate ground water, surface water and surrounding soils (Dobson & Moffat, 1994; Gordon *et al* 1989; Menser *et al* 1979). Sometimes collected leachate is recirculated and put back into the landfill in order to promote natural filtration and the microbial decontamination of the leachate (Menser *et al* 1983; Townsend *et al* 1994). The irrigation of landfills with leachate increases the moisture of the landfill, which can benefit the micro-organisms responsible for waste decomposition and stabilisation (Towsend, *et al* 1994) and help with plant moisture requirements (Maurice *et al* 1997). However, irrigation with leachate or when uncontrolled leachate contaminates cover soils it can have a negative effect on vegetation (Menser *et al* 1983; Tong & Wong 1984). This has been attributed to excessive salinity created by the leachate, thus causing osmotic and ionic stress in plants (Ettala, 1988, Cureton *et al*, 1991, Menser *et al*, 1983). Leachate conductivity generally ranges from 0.2 Sm^{-1} to 0.9 Sm^{-1}. An analysis of the effects of leachate indicate that leachate with an electrical conductivity between 0.2-0.4 Sm^{-1} tends to cause slight to moderate tree injury (Bradshaw & Chadwick, 1980). Leachate contamination of cover soils can also result in soil pH changes beyond the normal range (4.5 - 8) suitable for vegetation (McKendry, 1996). High values of particular elements in leachate can also have negative effects on plants. High levels of chloride can result in foliar chloride levels between 2000- 7000 mg kg^{-1} which is within the range of chloride toxicity resulting in symptoms such as leaf discoloration and leaf loss (Menser *et al* 1983; Ettala, 1988). High concentrations of heavy metals in leachate may also result in phytotoxicity. Rainfall and evaporation influence the effects of leachate. An increase in rainfall will result in leachate dilution and lower concentrations, which may be below levels of phytotoxity. However during drier seasons evaporation will result in higher concentrations and the potential for greater negative impacts.

The depth and quality of landfill cover materials is also an important factor determining the success of vegetation establishment on landfills. Completed landfill sites are usually clay capped and covered with a layer of topsoil. Areas of an operational site which have been out of use for any length of time are usually covered with waste soils, layered with topsoil and vegetated so as to aesthetically improve the site. The depth of the topsoil layer can influence the success of revegetation. Shallow soils are prone to waterlogging, desiccation and are also found to restrict the root growth, thus reducing nutrient uptake and anchorage (Dobson & Moffat, 1994, McKendry, 1996). Shallower soils are suitable for grasses and shrubs, which have shallow root systems. For a general vegetation cover 50-100mm soil depth is sufficient (Ettala *et al* 1988). However, when planting trees special consideration of soil depth needs to be made. Trees planted in shallow soils often die or have poor health, and due to insufficient anchorage, are susceptible to windthrow (Dobson & Moffat 1994). For the development of trees a minimum soil depth of 1m is recommended (Dobson & Moffat 1994; Gilman *et al* 1985). A soil depth greater than 2m would be considered unnecessary as the majority of trees roots do not penetrate more than 1.5m (Dobson & Moffat, 1994). A survey conducted by Ballardini and Lassini (1997) on 13 tree species growing on a landfill indicated that if the site was not sealed (clay capped) a topsoil layer of 1.5m could also be regarded as excessive, as landfill gas infiltration limited rooting depths.

Cover soils are not always easily available and are often expensive either due to actual cost, transport costs or both. The expense and availability of cover soils usually results in the utilisation of whatever soil is available at the time and the minimum possible amount is usually used. These soils frequently have poor structure and low nutrient content (Flower

et al 1981). Sometimes before a site becomes operational the original topsoil layer is removed and stored for the later restoration of the landfill. Unless this is done correctly i.e. stored in different horizons, handled only when dry, and not stored for excessive amounts of time, the quality of the soil rapidly deteriorates (McKendry, 1996; Williamson *et al* 1982). The nutrient content may be considerably reduced and the physical structure of good topsoil destroyed by poor handling practices (Williamson *et al* 1982; Cole *et al* 1978).

The most readily available soils are usually of poor quality, comprising a mixture of building rubble, stones, sands, clay and general unwanted soil material. The wastes that have been deposited are covered with soils so as to reduce smells, rodents and waste being blown off site. In order to get the maximum amount of waste into a landfill, specialised vehicles that are used to move the waste into position are designed also to compact the ground at the same time. The action of these vehicles and the general heavy vehicle traffic found on landfill sites results in a very high compaction of waste and cover soils.

The poor quality soil and the high degree of compaction results in poor soil structure for vegetation growth (Heilman, 1981; Insley & Carnell, 1982). A good soil should have sufficient coarse pores to facilitate soil aeration, downward drainage of excess water, and growth of plant roots. However, it is also essential to have sufficient fine pores to retain water. These properties of a soil are very vulnerable and can be destroyed by compaction during soil storage and mechanised earth moving, especially when wet. The living components of the soil, such as worms, fungi etc. which are important in developing and

maintaining structure and fertility tend to be the first to be affected in earth moving processes (Greacen & Sands, 1980). Thus soil compaction is a major consideration in successful tree growth on landfill sites because it is responsible for reduced pore space, aeration, water holding capacity and root penetration (Flower *et al* 1981; Greacen & Sands, 1980; Liang *et al* 1999). Bulk density is a measure of weight per unit volume oven dried soil and refers to the relationship between soil density and pore space. Plant root growth is found to decrease in compacted soils, with root growth decreasing in a linear manner in relation to bulk density (Heilman, 1981). Plant roots will rarely penetrate light textured soils with a bulk density greater than 1.7- 1.8 gcm $^{-3}$ or a heavy textured soil with a bulk density greater than 1.5- 1.6 gcm^{-3} (Dobson & Moffat, 1994). Guideline standards for the main soil variables, which are required for the establishment of trees on a landfill site are given in Table 1.4.

Table 1.4: Minimum standards for soil forming materials acceptable for woodland establishment on landfill sites.

Component	Minimum standard
Bulk density	<1.5 gcm $^{-3}$ to at least 50cm deep
Stoniness	<40% by volume with few stones greater than 100mm
pH	4.0-8.0
Electrical conductivity	< 0.2 Sm^{-1} (1:1 volume soil: water suspension)

Adapted from Moffat and Bending, 1992

The moisture of landfill soils is largely influenced by the degree of compaction. Compaction leads to a higher degree of run-off and less infiltration (Flower *et al* 1981; Greacen & Sands, 1980). However, the soil moisture of a landfill is generally lower than that of the same soil not on a landfill. This is attributed, at least in part, to the reduced capillary rise of water through the refuse and into the cover soils during dry periods. The refuse lacks the capillarity capacity needed for water movement found in normal soils. These periods of reduced moisture in the cover soils of landfill sites has been identified as a potential problem for some plant species in some situations (Gendebien *et al* 1992). The poor soil structure of landfill soil not only results in dry conditions but can also result in poor drainage and waterlogging. Waterlogging often occurs where there are large amounts of uncontrolled leachate production, which together with poor soil structure results in waterlogging and the development of anaerobic soil conditions (Dobson & Moffat, 1994).

Apart from soil variables there are other possible site-specific factors that maybe involved in limiting plant growth on landfills. Poor silvicultural practices and tree maintenance often play a large role in the success or failure of trees planted on landfill sites. Planting of trees by unqualified or poorly trained personnel, inappropriate planting stocks and ineffective weed control is often found to be the causes of failure in revegetation projects (Dobson & Moffat, 1994; Insley, 1980). Further disturbance may be caused by animals such as rats, moles and caterpillars which can be responsible for damage to plants.

The damage to vegetation after establishment is often a problem, especially if areas of the site are still in operation. The movement of heavy vehicles can result in accidental damage

to plants (Ettala, 1988). Operational landfill sites often require unplanned structural changes so as to control rainwater runoff or gas migration. Such alterations may disturb areas, which were vegetated. Vegetation to improve the poor aesthetics of an operational site will inevitably experience some kind of disturbance. The dust, produced by wind and movement of heavy vehicles, can cause the stomatal pores of plants to become blocked, thus, reducing transpiration and gaseous exchange. Large amounts of wind blown rubbish such as plastic bags can get caught in tree branches. For younger trees plastic and paper caught in their branches can result in the branches breaking and increase the possibility of windthrow. Landfill sites are often positioned near industrial areas where the emissions of phytotoxic gases such as sulphur dioxides or fluorides may be problematic. These emissions are known to effect the health of vegetation and could add to the stresses already presented by landfill conditions.

It is clear that a landfill environment has numerous factors that can limit the success of vegetation establishment. However, as with most activities that result in land degradation, the key to rehabilitation is through the use of suitable plant species. There is international literature which discusses plant species selection for landfills, however, no studies on indigenous South African species have been published. Research on suitable species for landfill revegetation appears to have been particularly productive in Hong Kong, United Kingdom, U.S.A and to a lesser extent in Finland (Table 1.5). Variability in species performance on landfills has been apparent to all researchers, with particular species having a greater tolerance to landfill conditions than others (Table 1.5). Even though the reasons for poor vegetation growth on landfills are relatively universal species tolerance to landfill conditions will be influenced by climatic differences, thus tolerant species selected

in anyone country may not be suitable for another country. The scope and need for further research, on a greater number of indigenous species from a wider range of geographic areas becomes apparent when one considers that landfilling is the predominant form of waste disposal in the world.

Table 1.5: A survey of plant species and their performance under landfill environmental conditions.

Species	Reported performance	Country	Reference
Abies alba	Tolerant to oxygen deficient soil, therefore may tolerate anaerobic landfill conditions.	United Kingdom.	Dobson & Moffat. 1994.
Abies spp	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Acacia confusa.	One of the most abundant tree species found in a survey of 13 landfills.	Hong Kong.	Chan *et al*, 1996.
	Suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Acer rubrum	Ranked 10th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Aesculus hippocastanum	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Ailanthus altissima	A predominant species naturally colonising 4 landfills in New York.	U.S.A.	Robinson *et al* 1992.
Albizzia lebbek	Suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Alnus glutinosa	Tolerant to landfill soils if aerobic for less than 0.5m.	United Kingdom.	Crook, 1992.
	Considered the most tolerant species when compared to *Prunus avium, Betula pendula, Fraxinus excelsior* and *Quercus robur*	United Kingdom	Mackay & Richardson. 1996
Alnus incana	Tolerant to landfill soils if aerobic for less than 0.5m.	United Kingdom.	Crook, 1992.
Aporusa chinensis	Not suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Betula pendula	Tolerant to landfill soils if aerobic for less than 0.5m	[illegible]	[illegible]
Salix aquatica	One of the most productive species growing on six landfills.	Southern Finland.	Ettala. 1988.
	Growth not influenced by high levels of CO_2 in landfill soils in three landfills.	Finland	Maurice *et al* 1997
Salix babylonica	Ranked 18th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Salix caprea	Non survived in 2 year experiment, even with a 0.5m clay or compost layer over the landfill cover material.	United Kingdom.	Moffat & Houston. 1991.
Salix spp.	Tolerant to landfill soils if aerobic for at least 0.5m.	United Kingdom.	Crook. 1992.
Salix viminalis	Damaged by leachate irrigation in a survey of six landfills.	Southern Finland.	Ettala. 1988.
	Growth not influenced by high levels of CO_2 in landfill soils in three landfills.	Finland	Maurice *et al* 1997
Taxus cuspitata	Ranked 2nd most tolerant to landfill conditions of the 19 species screened	U.S.A.	Flower *et al* 1981
Tilia americana	Ranked 8th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Tilia spp.	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Tristania conferta	Suitable for landfill revegetation.	Hong Kong	Chan *et al* 1991

1.4 AIMS AND OBJECTIVES

1.4.1 Research aims

The nature of landfill environmental conditions makes the successful establishment of vegetation on operational and complete sites difficult. The key focus of this research was on the revegetation problems associated with an uncapped operational site.

The specific aims of this investigation were to identify and quantify the key environmental factors limiting vegetation establishment on the Bisasar Road Landfill, and to assess the relative plant performance of some indigenous tree and grass species. Tree performance was experimentally investigated further both in the field and using a soil gas fumigation system. General physiological attributes were sought which could improve species selection for landfill revegetation.

1.4.2 Thesis structure

An investigation into the micro-distribution of grass species naturally colonising the Bisasar Road Landfill was conducted to assess plant species performance and possible limiting variables (Chapter 2). An investigation into an unsuccessful revegetation attempt, using indigenous tree species, on a stability berm at the Bisasar Road Landfill was conducted, and is described in Chapter 3. This provided preliminary information about the relative tolerance of a number of indigenous tree species and the key variables responsible for poor tree survival. Based on this work, a field experiment was designed in order to make a more detailed assessment of the suitability of 10 indigenous tree species for landfill revegetation. The field experiment also assessed the usefulness of a topsoil layer for improving the survival of trees and provided a comparison with direct planting in the

normal cover soil. This experiment provided an evaluation of some of the variables which limited tree growth and survival (Chapter 4).

In the field investigations and experiment the heterogeneity and dynamic nature of the landfill environment and the high mortality of less 'tolerant' species often made it difficult to explain differential species performance and establish the role of soil CO_2 and O_2 in determining plant health on the landfill. To provide an experimental approach, a soil gas fumigation system was designed and constructed. Using two tree species with different performance from the field experiment, the fumigation system was used to test the hypothesis that differential species performance on the landfill was due to elevated soil CO_2 and low O_2 (Chapter 5). The fumigation experiment aimed to evaluate the relative importance of high soil CO_2 and low O_2 concentrations in determining plant performance as well as the potential for antagonistic, additive or synergistic effects between these two variables.

A discussion of the main conclusions is given in each of the four separate result chapters. A final overall discussion of the results is given in Chapter 6, which considers: key limiting factors for vegetation establishment; plant species response and selection; and the objectives for further research.

1.5 SITE DESCRIPTION: THE BISASAR ROAD LANDFILL

The Bisasar Road Landfill site is situated in the Springfield area of Durban, South Africa. The 21 million cubic meter capacity site first started operation in 1980 and serves the waste disposal need of the city of Durban. The site is bounded to the north by the flood plains of the Umgeni River on which are sited the Clare Estates School and the Solid

Waste, Health and Electricity Departments of the City of Durban. Residential areas along Kennedy, Clare, Burnwood and Dhulam Roads bound the site to the east, south and west. In the south-eastern corner lies the City of Durban Nursery (Figure 1.1). The landfill is located within a north facing, steep sided valley with its floor situated approximately 12m above sea level on the Umgeni river flood plain and the top of the valley situated to the south at approximately 110m above sea level (Figure 2.1). The landfill does not have a clay or geotextile liner.

The underlying geology of the area consists of the Pietermaritzburg Formation of the Ecca Group in the Karoo Sequence. The Pietermaritzburg Formation is extensively intruded by Dolerite sills and dykes of the Jurassic Age and is comprised of predominantly bedded, dark grey to black, carbonaceous shales and micaceous siltstones with occasional bands of thin sandstone. The dolerite intrusions are usually extensively weathered to a yellow orange and reddish brown silty clay. A major Dolerite sill occurs within the eastern side of the valley in which the site is located. Several geological faults are found approximately 500m to the east and the west of the site (Loudon and Partners, 1993).

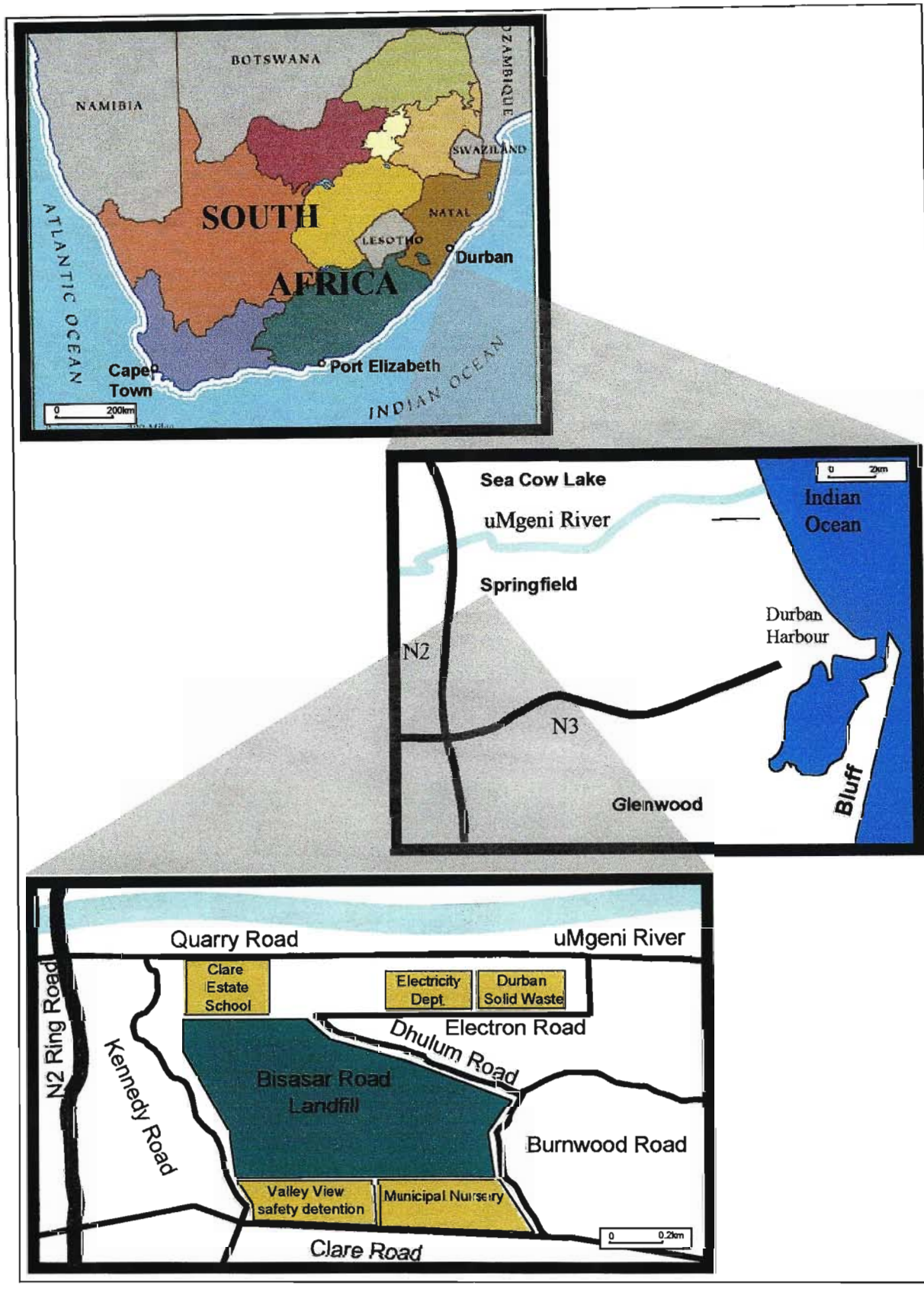


Figure 1.1: Location of Bisasar Road Landfill in Durban, South Africa

The Bisasar Road landfill site is used for the disposal of domestic and general industrial waste. The site is located in the centre of the municipal area, thus minimising the cost of transporting waste for the city, private individuals, contractors and other local authorities. The landfill site serves mainly the Greater Durban Metropolitan Area disposing of approximately 48862 tons of mixed waste on a monthly basis. A break down of the types of waste disposed of can be seen in Table 1.6.

The landfill operation is structured in a series of terraces of waste, which are compacted and covered with waste soils and rubble at the end of each day. These terraces are worked forward until they reach a main stability berm at the base of each main terrace. Each phase of development will have a main containment or stability berm. This berm is usually built with an initial lift of 5m and thereafter lifts of 2m with each set of terraces until the designed filling level of the site is reached. The berm is constructed with non-compactable material such as metal, rock, builders rubble or reinforced concrete. The final outer slope of the berm is then top soiled and vegetated.

Table 1.6: Receipt of waste (average per month, in tons) at Bisasar Road landfill site (Adapted from Lombard & Associates,1994)

Waste types	Monthly Average (tons)
Domestic	13861
Trade	12783
Garden	9143
Cover	11821
Street sweeping	1135
Vehicles	16
Fish	41
Fresh produce	61
Other	1
Total	48862

Annual rainfall figures from the meteorological station at Durban International Airport, 20km south of Bisasar Road landfill, are shown in Table 1.7. The minimum and maximum average summer and winter temperatures are 19 - 26°C and 12 - 23 °C respectively (Michellin, 1990).

At the time of the preliminary investigation (1996) the northern side of the site, directly behind the main stability berm, was not in use, however, the installation of gas reclamation wells was planned for this area (Figure 1.2). The project involved the sinking of 24 wells for the extraction of methane from the underlying decomposing waste, to be used on a commercial scale. Waste disposal was continuing mainly on the southern section of the site which receives approximately 48900 tons of waste per month (Table 1.6).

There has not been any establishment of indigenous woody vegetation on the Bisasar Road Landfill. On the site, and the surrounding disturbed areas alien, invader species such as *Ricinus communis* (castor oil bush), *Solanum mauritianum* (bugweed), *Melia azedarach* (syringa) and other exotics are the main species that occur. The south eastern and north western corners of the site remain permanently wet due to a natural spring and species such as *Phragmites* spp (common reed), and *Bambusa* spp (Bamboo), *Typha* spp (bulrush) are found. The grass cover on the landfill is mainly *Cynodon dactylon,* interspersed with numerous young *Melia azadarach* which have established themselves in areas which have not been utilised for waste disposal for any length of time.

Table 1.7: Average rainfall figures for the year as recorded by the meteorological station at Durban International Airport (1950-1995).

Month	Average Rainfall (mm)	Max. Rainfall (mm)
January	135	310 in 1984
February	126	361 in 1986
March	127	397 in 1976
April	86	283 in 1957
May	60	227 in 1971
June	27	139 in 1961
July	34	147 in 1963
August	59	252 in 1981
September	77	402 in 1987
October	103	251 in 1964
November	112	246 in 1989
December	104	331 in 1958
Summer (Oct. - Mar.)	707	
Winter (Apr. – Sep.)	343	
Total	1050	

CHAPTER 2: THE MICRODISTRIBUTION OF GRASSES FROM VOLUNTEER COLONISATION

2.1 INTRODUCTION

The establishment of vegetation on recently covered landfills is very important for the stabilisation of soils and prevention of erosion (Gilman *et al* 1985). However, many landfills experience stunted vegetation growth and poor cover including bare patches where plants do not grow, thus not achieving either the stabilisation of cover material or the improved amenity value intended (Lan & Wong, 1994; Davis & Coppeard, 1989; Wong, 1988). Poor plant growth on landfills has been attributed to high concentrations of carbon dioxide and methane, low amounts of oxygen, poor soil structure and low soil nutrient availability, all factors that commonly occur in landfill soils (Gilman *et al* 1985).

Grasses tend to survive better than other plant types such as trees and shrubs on landfills, especially where there are high soil concentrations of carbon dioxide and methane (Lan & Wong, 1994). The better survival of grasses has been explained by their shallow rooting depths. The roots remain near the surface and thus avoid the higher concentrations of carbon dioxide and methane experienced at depth in the soil of landfills (Lan & Wong, 1994). Erosion by wind and water is effectively controlled by the closed leaf canopy, relatively high basal cover, and fibrous root systems provided by grasses. They also form a useful 'pioneer' community which may facilitate the development of a more complex vegetation structure on landfills (Zacharias, 1995). Grassland forms a key vegetation type in the revegetation of operational sites and the rehabilitation of landfills into after uses such as parks, gardens and golf courses.

The aim of this investigation was to identify the environmental factors limiting grass growth in certain areas of the Bisasar Road landfill, possibly providing insight into ameliorative procedures needed to achieve a more complete ground cover. The identification of species preferences as to microhabitat conditions sought to identify species relatively more tolerant to landfill conditions.

2.2 MATERIALS AND METHODS

2.2.1 Site description

A temporarily complete section of the Bisasar Road Landfill, Springfield, Durban, South Africa, was naturally colonised by a variety of grass species over an approximate eighteen month period. This section of the site was not clay capped, as waste filling was likely to continue in this area during the future years of the landfill life span. The grass was growing in a 500mm waste soil layer which formed the cover over an approximately 30m depth of domestic waste, filled into the valley since 1989, and which formed a large terrace. This vegetation dominated by grasses had a patchy appearance with bare areas where no vegetation had colonised (Figure 2.1).

Figure 2.1: The vegetation dominated by grasses had a patchy appearance with bare areas that no plants had colonised.

2.2.2 Sampling design and field measurements

Within a temporarily complete section of the Bisasar Road landfill (approximately 15000m^2), four conspicuous patches without grass were selected. The four patches were selected outside of the effective range of the area identified for gas reclamation well installation (Dorkin, D. 1996 *pers comm*). This was to ensure that landfill gas, a potential factor causing the patches, was not altered during the investigation. The area of each of the four bare patches was divided into quarters. Within each quarter a random transect radiating out from the centre of the bare patch into the surrounding vegetation was positioned. Three 0.5m by 0.5m quadrats were placed along each transect, one within the bare patch (no grass); another incorporating the first grasses on the border of the patch (border grass); and

the final quadrat positioned within the first well established stand of grass (established grass). Grass and environmental variables were measured within each quadrat.

The different grass species present within each quadrat were identified. The above ground plant material (live standing crop) for all species was collected from each quadrat and oven dried at 105°C until a constant weight. This was used to calculate species biomass and total biomass for each quadrat (Allen, 1989).

A 400mm long, 22mm outer and 15mm inner diameter plastic pipe was used for gas sampling. The end 200mm of the pipe was perforated with sixteen 5mm diameter holes and inserted 300mm below the soil surface in each quadrat. A more complete description of the gas sampling pipe is described in Chapter 3, however, it differs in length by 600mm. Due to the high compaction and large stone content, the hole in the ground into which the gas samplers were inserted had to be drilled with a 38mm masonry bit. Although the drill bit had a greater diameter than the sampling pipe the slight subsidence of the hole wall after drilling resulted in a close fit between the hole and sampling pipe. The gas samplers, once inserted into the hole, were tightly packed into the ground and sealed with airtight caps. They were allowed one week to equilibrate with the soil atmosphere before percentage methane, carbon dioxide and oxygen in air were measured using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. The soil temperature at a 200mm depth for each quadrat was recorded by inserting a digital thermometer (YFE YF-1062) into each gas sampler.

2.2.3 Soil analysis

A soil sample was collected from the surface to a depth of 150mm from each quadrat. The single sample from each quadrat was immediately sealed into a plastic bag and then mixed. Two sub-samples of soil from each quadrat were analysed for percentage moisture content by oven drying at 105°C (Grimshaw, 1989). A sub-sample of soil from each quadrat was sent to the Kwazulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: extractable P; K; Ca; Mg; Zn; Mn; sample density; extractable acidity (titrated NaOH expressed as centimoles of acidity per litre of soil); pH; % organic carbon; % clay (Hunter, 1974). A description of the techniques used for these analyses is provided in Chapter 4 (section 4.2.4).

The remainder of the soil was air dried and passed through a 2mm sieve, separating the soil from the stone. The stone content was then calculated as a percentage weight of the original sieved sample. For conductivity measurements approximately 20g of sieved soil was saturated with de-ionized water and allowed to stand for 24 hours. The high clay content of the soil samples made it difficult to extract any filtrate using a Buchner funnel and filter paper under suction with a vacuum pump, therefore, centrifugation was used instead. The soil water mixture was centrifuged using a Beckman G.P. centrifuge (No. 355953) at 3700rpm (relative centrifugal force = 2127.4) for 30 minutes to extract the supernatant of which the conductivity was measured using a Crison MicroCM 2201 conductivity meter corrected to 25 °C.

Statistical analysis of the data collected was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data was analysed using analysis of variance. If there was a

significant difference ($p<0.05$) in data with more than two samples a Sheffe Multiple Range test was performed to determine which differences were significant ($p<0.05$). The relationship between grass biomass and the environmental conditions measured was also evaluated using a scatter plots and Pearson's Product-moment Correlation Analysis.

2.2.4 Bioassay

A soil sample (approximately 2kg) taken from each quadrat on the landfill was air-dried and sieved with a 2mm sieve. Decomposing waste material below the soil cover of the area investigated would be the main cause of potentially high carbon dioxide, methane and low oxygen in the soil. Therefore, the removal of the soil samples from the site would change these conditions, which could be effecting plant growth, thus, returning the normal gas composition to the soil atmosphere. Further, sieving of the soil removed the stones and altered the original field structure of the soil, thus improving the physical aspects of the soil which might be causing poor grass growth in the field.

The sieved soil samples from each quadrat were placed into 350ml plastic containers (Container Corporation) with holes drilled in the bottom. Stolons from a single *Cynodon dactylon* parent plant were cultivated in seedling trays in a glass house for 3 weeks. The resultant genetically similar plants of similar size were selected and the shoots were trimmed to the same height. Twenty of these plants were randomly selected and oven dried at 105°C so as to provide a figure for the original mean root and shoot weight of the plants to be used in the bioassay. Forty-eight plants were then planted into the plastic containers giving a single plant in each container, which contained soil from a particular quadrat. This gave four replicate plants for each of the three areas (no grass, border grass and established grass) of

each patch, totalling 12 plants for each of the 4 patches and 48 plants/containers in all. So as to provide a control another 6 plants were put into similar containers containing potting soil. The plants were grown for 4 weeks under random block design in a controlled environment chamber (Conviron), provided with 12 hours light at 25°C, 12 hours dark at 18°C and watered once a week ensuring that the soil remained moist.

If the causal environmental conditions in the bare areas on the landfill were resulting in a chronic response in plants, then some short term but permanent attempt at colonisation of the bare areas would be apparent. Therefore, the bare areas would not be totally void of vegetation but would be characterised by stunted and sickly young plants attempting to colonise the area. Considering the areas without grass on the landfill had no vegetation at all it was assumed that the causal environmental conditions was resulting in an acute response in plants and thus no vegetation growth was found. Therefore, a one month period for the bioassay was thought to be sufficient to elicit a detectable response in the grass planted.

After the four week period the plants and soil were carefully removed from the containers and the soil washed from the roots. The plants were then oven dried at 105° C until a constant weight. The dry weight of the roots and shoots of each plant was then measured. The root and shoot weights of the four plants grown in the soil from the each of the four quadrats in each area, namely the no grass area, border grass area and established grass, of each patch were compared using an analysis of variance. The root and shoot mass data from the different areas for the four patches were then pooled together (n=16) and again analysed using an analysis of variance and Sheffe Multiple Range test.

2.3 RESULTS

2.3.1 Field sampling

Twelve different species of grasses were identified in the area of investigation on the Bisasar Road Landfill Site. These were found with different relative abundances and distributions (Table 2.1). *Paspalum paspalodes distichum, Cynodon dactylon, Sporobolus africanus* and *Panicum maximum* were the most common species with the highest frequency in the quadrats with grass and with the highest overall standing crop. *Paspalum paspalodes* was only found in patches 1 and 2, but had a relatively large biomass in the border areas in comparison to the other species (Table 2.1). Similarly, *Sporobolus africanus* had its highest biomass in the border areas of all three of the patches in which it was present. *Panicum maximum* was only found in patches 3 and 4, and had a relatively larger biomass in the established stands of grass, especially in patch 4. *Cynodon dactylon* was the most abundant species in terms of biomass and frequency and was found in all patches. However, the absence or relatively low biomass of *Cynodon dactylon* in border areas in comparison to the well-established stands of grass was apparent. These results showed that *Paspalum paspalodes* and *Sporobolus africanus* were the main species found in the borders of the areas where grass did not grow, whilst *Cynodon dactylon* and *Panicum maximum* were predominantly found in the established stands.

Many of the species were only found in one or two quadrats and had relatively low total biomass making it difficult to make any conclusions about their distribution other than that they were relatively uncommon species. However, it was noted that six of the twelve species were only found in border areas (Table 2.1). These species were *Chloris gayana, Digitaria eriantha, Echinichloa colona, Eragrostis curvula, Paspalum urvillei,* and

Sorghum bicolour. All of the six other species were found in both the established stands and the border areas (Table 2.1). It was clear that the border areas had higher species diversity in comparison to the established stands.

Table 2.1: Mean above ground biomass (dry mass (g) / 0.25m^2) of each grass species for the border area (B) and surrounding established grass (EG) for the four patches investigated on the Bisasar Road Landfill site.[1]

Grass Species	Patch1		Patch 2		Patch 3		Patch 4	
	B	**EG**	**B**	**EG**	**B**	**EG**	**B**	**EG**
Chloris gayana	6.5[1]	0	0	0	0	0	0	0
Cynodon dactylon	0	62.8	0	276.8	4.1	104.6	11.7	55.9
Dactyloctenium	0	2.4	0	0	0	0	0.4	0
Digitaria eriantha	4.6	0	0	0	0	0	0	0
Echinichloa colona	0	0	0	0	0	0	1.0	0
Eragrostis curvula	0	0	0	0	5.8	0	0	0
Melinis repens	0	0	0	0	0.2	0	0	4.1
Panicum maximum	0	0	0	0	1.5	19.3	0	62.2
Paspalum paspalodes	70.3	190.3	47.6	0	0	0	0	0
Paspalum urvillei	0	0	11.9	0	0	0	0	0
Sorghum bicolour	0	0	0	0	0	0	0.9	0
Sporobolus africanus	0	0	8.5	0	44.4	2.2	10.8	10.1

[1]The biomass is the mean of four quadrats in each area for each patch.

The data collected from each of the patches was pooled together in order to increase the sample size and decrease the effect of extreme values. With a larger sample size, significant changes in environmental variables were not as easily masked. Thus, significant differences in environmental variables, which may be a common cause for poor grass growth, could be identified. The pooled data was analysed using an analysis of variance and Sheffe multiple range test (Table 2.2). The environmental variables from all the patches and quadrats were also analysed in relation to the total biomass in each quadrat using a Pearson's product-moment correlation analysis (Table 2.4).

Table 2.2: Soil variables (mean and standard error; n=16) measured in no grass, border grass, and established stands of grass for combined data of the four patches.

Environmental variables	No grass	Border grass	Established grass
Oven dry Biomass (g)	0.0 ±0.0 a [1]	59.8 ±8.5 b	197.7 ±26.1 c
Extractable P (mg kg^{-1})	11.7 ±1.1 a	8.5 ±0.7 ab	8.7 ±0.7 b
Extractable K (mg kg^{-1})	231.7 ±32.2 a	255.9 ±49.7 a	304.2 ±65.7 a
Extractable Ca (mg kg^{-1})	1703.8 ±160.5 a	1222.7 ±100.0 b	1163.8 ±135.1 b
Extractable Mg (mg kg^{-1})	263.4 ±23.9 a	278.1 ±32.7 ab	424.6 ±61.4 b
Ext. Acidity (Cmol kg^{-1})	0.1 ±0.01 a	0.1 ±0.01 a	0.1 ±0.01 a
pH	7.6 ±0.1a	7.7 ±0.1 a	7.8 ±0.06 a
Extractable Zn (mg kg^{-1})	14.5 ±2.3 a	10.0 ±1.2 ab	8.5 ±1 b
Extractable Mn (mg kg^{-1})	45.6 ±9.1 a	35.3 ±6.0 a	52.5 ±9.9 a
Organic carbon (%)	4.4 ±0.2 a	5.1 ±0.4 a	4.2 ±0.3 a
Clay (%)	37.6 ±1.8 a	37.6 ±1.7 a	37.2 ±2.2 a
Moisture (%)	14.2 ±0.6 a	17.6 ±0.9 b	17.7 ±1.1 b
Stone (% weight)	57.0 ±2.6 a	52.6 ±2.2 a	52.5 ±3.7 a
Conductivity ($mScm^{-1}$)	5.2 ±0.4 a	5.9 ±1.0 a	5.2 ± 0.9 a
Methane (%)	17.5 ±4.3 a	15.9 ±4.5 a	8.5 ± 4.0 a
Carbon dioxide (%)	14.5 ±3.2 a	12.4 ±2.9 a	6.8 ±2.3 a
Oxygen (%)	12.4 ±1.6 a	12.4 ±1.6 a	15.5 ±1.6 a
Soil temperature (°C)	25.1 ±0.5 a	24.6 ±0.4 a	23.8 ±0.5 a

[1] The means in the rows across the table followed by different letters are significantly ($p<0.05$) different with a Sheffe Multiple Range test.

The pooled data showed significantly ($p<0.05$) higher concentrations of Zn, P and Ca in the no grass areas in comparison to the established stands with intermediate concentrations within the border area (Table 2.2). However, the Ca concentrations in the border areas were the same as the established grass area. The no grass area was significantly ($p<0.05$) lower in Mg and soil moisture in comparison with the established grass stand. Mg levels were intermediate in the border areas, however, there was no significant ($p>0.05$) difference in soil moisture between the border area and the established stands (Table 2.2).

It is important to note that although there was no significant variation in conductivity, methane, and carbon dioxide concentrations within the patches, the values measured in soil throughout the patches were beyond the normal range expected for healthy soils (Table 2.2).

When the data from each of the individual patches was analysed separately, other significant differences, which were not found with the analysis of the pooled data, were found, these included K, carbon content, Mn, and conductivity (Table 2.3). This suggested that these differences were probably patch specific and were not common for all bare patches.

Table 2.3: Soil variables (mean and standard error; n=4) which had significantly different values measured in no grass, border grass, and established grass in each of the four individual patches.

Soil variables	No grass	Border grass	Established grass
Patch 1			
Extractable Zn (mg kg^{-1})	27.4 ±4.5 a	11.5 ±3.3 b	11.0 ±2.7 b
Extractable K (mg kg^{-1})	284.4 ±33.2 a	278.5 ±41.7 a	478.7 ±82.0 b
Extractable Mn (mg kg^{-1})	33.0 ±3.7 a	59.6 ±13.9 ab	85.6 ±8.1 b
Moisture (%)	12.6 ±0.6 a	21.0 ±0.6 b	21.4 ±1.3 b
Patch 2			
Extractable Mn (mg kg^{-1})	48.2 ±4.6 a	49.3 ±1.4 a	92.2 ±9.1 b
Organic carbon (%)	4.2 ±0.2 a	6.5 ±0.7 b	4.0 ±0.2 a
Patch 3			
Extractable P (mg kg^{-1})	16.4 ±3.1 a	9.2 ±1.8 ab	7.1 ±1.4 b
Patch 4			
Extractable Mg (mg kg^{-1})	150.4 ±17.4 a	216.2 ±33.9 ab	325.2 ±51.5 b
Conductivity (mS/cm)	5.7 ±0.5 a	2.2 ±0.6 b	1.8 ±0.3 b

[1] The means in the rows across the table followed by different letters are significantly different with a Sheffe Multiple Range test.

[2] Significance level, a $p<0.05$; aa $p<0.01$

Using the pooled data the relationship between the total biomass and the variables measured was analysed using a Pearson's Product-moment correlation. The variables which showed a significant correlation ($p<0.05$) were further analysed using a linear regression analysis (Table 2.4).

Table 2.4: Correlation between soil variables measured and the total plant standing crop of the individual quadrats from all of the patches (n=48). The relationship between the variables with significant ($p<0.05$) correlation coefficients was analysed using a linear regression and the R-squared value and the level of significance given.

Environmental variable	Correlation coefficient	Linear regression R-squared value (%)
Phosphate (P)	-0.15	
Potassium (K)	0.37* [1]	13.49**
Calcium (Ca)	-0.47**	21.83**
Magnesium (Mg)	0.62**	37.24**
Exchangeable acidity	-0.22	
pH	0.32*	9.96*
Zinc (Zn)	-0.30*	8.83*
Manganese (Mn)	0.40**	15.24**
Organic carbon	-0.16	
Clay	0.06	
Moisture	0.41**	16.41**
Stone content	-0.21	
Conductivity	0.16	
Methane	-0.23	
Carbon dioxide	-0.30*	9.07*
Oxygen	0.24	
Temperature	-0.46**	20.89**

[1] Significance level * $p<0.05$; ** $p<0.01$

The results for the levels of soil Mg and moisture showed a positive relationship with total biomass (Table 2.4) reinforcing the results indicated by the ANOVA (Table 2.2). There was also a significant ($p<0.05$) positive correlation between K concentrations and grass biomass however, the ANOVA results suggested that K variations were patch specific. Although a

relationship between low soil K and low grass biomass was apparent, the discrepancy between the correlation and ANOVA results made it difficult to determine the importance of soil K in determining grass establishment.

The Zn and Ca concentrations had a significant negative relationship with total biomass (Table 2.4) similar to the ANOVA results. It is interesting to note that the highest r-squared values for the linear regression were for Mg and Ca, suggesting that a relatively large percentage of the variability in biomass was determined by the concentrations of Mg and Ca in the soil. It is also interesting to note that whilst Mg had a positive relationship with grass biomass, Ca had a negative relationship, possibly suggesting that the relationship between soil Ca and Mg could influence the growth of grasses. To asses this the analysis of the ratio Ca/Mg to grass biomass showed a significant ($p<0.01$) negative linear relationship ($R^2 = 0.136$) and a significant ($p<0.01$) negative correlation coefficient (-0.369). However, the correlation coefficient and R^2 value for the relationship between Ca/Mg and grass biomass was less than that of either of the individual nutrients separately (Table 2.4). This indicated that the absolute levels of Ca and Mg in the soil were probably more important, in terms of grass biomass, than the relationship between the two variables.

The results of the correlation analysis for soil organic carbon content, conductivity, methane, oxygen, stone content, % clay, exchangeable acidity and grass biomass further reinforced the ANOVA results showing that there was no relationship between grass biomass and these variables. However, the correlation analysis showed a positive relationship between soil Mn levels and grass biomass yet there were no significant differences between the quadrats with the ANOVA. Similarly the ANOVA indicated that there was a relationship between grass growth and P concentrations yet there was no

significant relationship shown with the correlation analysis. Thus, the variations in Mn and P were difficult to interpret and their relationship with grass growth was unclear. However, similarly to K, the unclear results made it difficult to determine the if the variation in soil Mn and P were likely to explain the lack of grass establishment.

The correlation analysis revealed some relationships between biomass and soil variables that were not identified by the ANOVA. These included soil pH, temperature and carbon dioxide concentration. Soil pH had a significant ($p<0.05$) positive relationship with grass biomass, suggesting that higher grass biomass was found in areas with a higher soil pH. Soil temperature had a significant ($p<0.01$) negative relationship with grass biomass. The higher soil temperature in the no grass areas was probably due to the lack of vegetation, therefore there was greater heating of the soil by the sun. This probably indicates that the soil temperature relationship was more a symptom than a cause of poor grass establishment. It could also have been due to the infiltration of warm landfill gases as indicated by the significant relationship between carbon dioxide and grass biomass. Carbon dioxide had a significant ($p<0.05$) negative relationship with grass biomass, suggesting that higher levels of carbon dioxide were associated with lower grass biomasses. Carbon dioxide concentrations in the soil ranged from 0% to 39% with generally a lower grass biomass at higher gas concentrations (Figure 2.2).

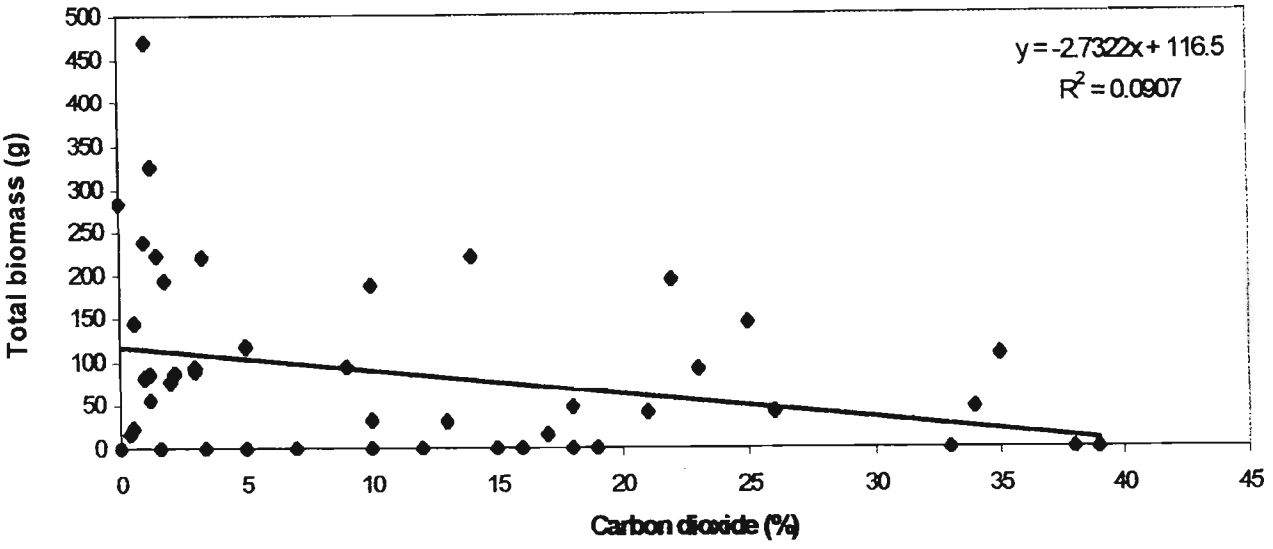


Figure 2.2: Linear regression ($p<0.05$) of the relationship between carbon dioxide and total above ground biomass.

In summary, it appeared that of the variables measured, the higher levels of Mg, moisture and K in landfill cover material were associated with higher grass biomass, whilst high levels of Zn and Ca were associated with low biomass. There was also evidence indicating higher grass biomass was associated with higher pH values and the bare areas were associated with high soil temperature and elevated soil CO_2 concentrations. The data suggested that there may be some relationship between grass growth and soil Mn and P levels however, the results were unclear.

The grass species that were found in more than three quadrats had sufficient data to be subjected to a correlation analysis to determine the relationship between the environmental conditions and the biomass of individual species (Table 2.5). However, with low sample sizes (3 quadrats) the chance of making a type two error is greater than with a higher number of replicates. *Panicum maximum* was only found in four quadrats, therefore, the

probability of correctly rejecting the null hypothesis was low. However, *Cynodon dactylon, Sporobolus africanus* and *Paspalum paspalodes* were found in a larger number of quadrats, namely 14, 8, and 10 respectively, thus increasing the power of the analysis.

Table 2.5: Results of a correlation analysis for the biomass of *Cynodon dactylon, Paspalum paspalodes* and *Sporobolus africanus* for the environmental variables measured

Environmental variables	Grass Species		
	Cynodon dactylon (n=14)	*Paspalum paspalodes* (n=10)	*Sporobolus* (n=8)
Extrac. Phosphate (P)	0.03	0.23	0.27
Extrac Potassium (K)	0.47	0.56* [1]	-0.08
Extrac Calcium (Ca)	-0.49*	-0.52	-0.40
Extrac Magnesium (Mg)	0.78****	0.50	-0.37
Extractable acidity	0.14	-0.42	0.06
pH	0.36	0.29	0.25
Extrac Zinc (Zn)	-0.39	0.22	-0.27
Extrac Manganese (Mn)	0.64***	0.59*	0.17
Organic carbon %	-0.28	-0.54	-0.17
Clay %	0.29	-0.24	-0.04
Moisture	0.12	0.56*	-0.07
Stone content	-0.004	-0.07	0.094
Conductivity	0.15	0.05	-0.07
Methane	-0.50*	-0.16	0.64*
Carbon dioxide	-0.52*	-0.40	0.66*
Oxygen	0.52*	0.21	-0.65*
Temperature	-0.44	-0.58	-0.11
Total biomass of quadrat [2]	0.93****	0.99****	-0.057

[1] Significance level * p<0.1; **p<0.05; ***p<0.02; ****p<0.01

[2] Correlation between the individual species biomass and biomass of all the grasses in the quadrat.

The biomass of *Cynodon dactylon* had a significant positive correlation with the concentrations of Mg and Mn in the soil, p<0.01, p<0.02 respectively (Table 2.5). There were no other significant correlation using the probability level of p<0.05. However, the analysis of the results using the p<0.1 revealed a number of less obvious trends in the data.

Although the level of significance was lower than the commonly accepted limit ($p<0.05$) it provided insight into the possible relationships between the variables measured. Higher concentrations of Ca were found to be negatively correlated ($p<0.1$) with the biomass of *Cynodon dactylon* as found with the pooled species results. The biomass of *Cynodon dactylon* had a significant ($p<0.1$) negative correlation with concentrations of carbon dioxide and methane, however, the biomass increased with increasing concentrations of oxygen ($p<0.1$). The results suggested that *Cynodon dactylon* was growing better in areas with lower carbon dioxide and methane concentrations but higher oxygen concentrations. (Table 2.5). However, the fact that the level of significance ($p<0.1$) for the correlation between biomass of *Cynodon dactylon* and Ca, carbon dioxide, methane, oxygen was very low, limits the interpretation of the correlations to only suggestions rather than reliable conclusions.

Paspalum paspalodes was the only other species to have any significant correlation between biomass and nutrient concentrations in the soil. Concentrations of Mn and K were positively correlated ($p<0.1$) to the biomass of *Paspalum paspalodes* ($p<0.1$). This suggested that variations in the soil nutrients maybe a factor limiting the success of some grass species. The biomass of *Paspalum paspalodes* was also found to significantly increase ($p<0.1$) with the moisture content of the soil, suggesting a possible affinity for moist areas. The level of significance ($p<0.1$) again was very low for the correlation, thus, suggestions rather than conclusions could be made.

Sporobolus africanus had a positive correlation ($p<0.1$) between biomass and carbon dioxide and methane, but a negative correlation ($p<0.1$) with oxygen. This suggested that *Sporobolus africanus* was mainly growing well in areas that had high concentration of

methane and carbon dioxide and low oxygen. This does not necessarily lead to the conclusion that *Sporobolus africamus* preferred these conditions. The reduced competition caused by reduced *Cynodon dactylon* biomass possibly provided an area for establishment.

The correlation between individual species biomass and total biomass gives an indication of the degree to which the species dominates the growth or possibly the amount of competition which the individual species was being exposed to (Table 2.5). *Cynodon dactylon* and *Papsalum paspalodes* have a highly significantly positive correlation ($p<0.01$) with the total biomass, indicating that they are the dominant species in the quadrat. *Sporobolus africamus*, has no significant correlation with total biomass indicating that this species growth is independent of how well other species grow and does not become the dominant species itself (Table 2.5). This lead to the conclusion that *Sporobolus africamus* was perhaps less competitive than the other species but possibly less susceptible to high carbon dioxide and methane, thus allowing the species to grow in areas of higher carbon dioxide and methane.

2.3.2 Bioassay

Any significant difference in growth of the plants between the different soil samples in the bioassay could be attributed largely to soil chemical differences. This would indicate that the cause of the patchy grass growth on the landfill was connected to the chemical factors in the soil and not entirely caused by soil physical structure or landfill gas.

A significant ($p<0.01$) 50% increase in the overall average plant mass was evident for those plants grown in the bioassay for one month. This showed that sufficient growth had

occurred for any acute soil effects on grass growth in the bioassay to be detected. There were no plant mortalities indicating that none of the soil samples taken from the landfill had sufficiently severe toxicity or deficiency of trace elements to result in grass death in 1 month. The root and shoot mass of *Cynodon dactylon* grown in the soil samples from the quadrats in the different areas of each patch (the area without grass; the border grass area; and the surrounding established stand of grass) were compared using an analysis of variance. No significant differences ($p>0.05$) were found in root or shoot mass for any of the soil samples from the four patches investigated. When the data from the four patches were pooled together there was still no significant difference between the different areas from which soil samples were taken (Table 2.6).

Table 2.6: The mean root and shoot weight increase (± standard error) in a 4 week growth period, for *Cynodon dactylon* grown in soil samples from different areas of the four patches on the landfill. (Relative growth was expressed as the weight after 4 weeks minus the original mean weight calculated before bioassay).

Plant material	No grass	Border grass	Established grass	Control (potting soil)
Root mass	0.086±0.021	0.091±0.033 [a]	0.046±0.009	0.131±0.031
Shoot mass	0.200±0.033	0.200±0.034 [a]	0.180±0.037	0.257±0.055

[a] the means in the rows are not significantly different ($p>0.05$) with a Sheffe multiple range test.

These results suggest that soil chemical composition, especially nutrient availability or chemical toxicity by trace elements, was not responsible for the lack of grass growth observed in the four different patches investigated on the Bisasar road landfill. Therefore, the cause for the bare patches on the landfill may be due to one of the variables 'removed' when the soil samples were taken from the landfill, air dried and sieved. These would include changes in the soil atmosphere and in particular carbon dioxide, methane and

oxygen concentrations as well as changes in soil moisture, soil temperature and stone content.

Considering that stone content was not found to vary significantly on the site, nor was it significantly correlated with the differences in grass biomass sampled, it is unlikely to be the cause. The difference in soil moisture was attributed to evaporation due to the lack of grass cover (i.e. a symptom and not a cause of low biomass). Therefore, the bioassay highlighted the importance of the correlation analysis results that suggested that soil gas composition was influencing grass biomass and species distribution and suggested that soil nutrient composition was a less important determinant.

2.4 DISCUSSION

It is important to note that significant differences in the environmental variables measured between the no grass, border grass and established stands of grass do not necessarily identify the reason for the lack of grass growth in any particular patch. Any measured differences maybe due to substrate variation, or maybe as a result of the vegetation growing in the soil thus changing the soil characteristics. However, the comparison of the levels of the variables measured with normal soil conditions, as well as with the result of the bioassay, would help confirm the role these environmental variables had in affecting grass establishment and growth. The results provide an indication of which environmental variables do vary on the landfill and their possible relationship with grass distribution.

Twelve different species of grass were identified on the Bisasar Road Landfill site. This is similar to the grass species diversity found on the Gin Drinkers' Bay Landfill, Hong Kong on which 10 different grass species were recorded (Wong & Yu, 1989). A wide range of

cover materials used on landfills will influence the cover and number of species, and may result in high plant species richness (Ettala *et al* 1988). The types of waste underlying the cover material, which produce different amounts of landfill gas and leachate, may also cause variation in the soil, which also influences species colonisation and distribution.

Out of the twelve species, *Cynodon dactylon, Paspalum paspalodes* and *Sporobolus africanus*, in terms of relative abundance, were the most successful colonisers of this area of the landfill. The micro-distribution of the species around bare patches provided insight into the performance of the species in relation to possible spatially variable soil conditions. *Cynodon dactylon* was the predominant and most competitive species in the established stands, forming an almost complete monoculture. However, it appeared to be sensitive to the environmental variables causing the bare patches and was relatively less abundant in the border areas of the bare patches. The opposite was apparent for *Paspalum paspalodes* and *Sporobolus africanus*, although, not as widely distributed as *Cynodon dactylon*, these species were predominantly found in the border areas of the bare patches.

In the established grass stands the environmental conditions were sufficient to support a large standing biomass of *Cynodon dactylon* and competition between species was probably a major factor determining the distribution of other species. The reduced biomass in the border areas of the bare patches resulted in lower levels of competition and an opportunity for other species to colonise, thus resulting in a higher species richness. However, the species that colonised the border areas would have to be less sensitive to the environmental conditions causing the bare patches than *Cynodon dactylon*. Therefore, *Paspalum paspalodes* and *Sporobolus africanus*, which were the most successful colonises of the

border areas, probably had the greatest relative tolerance to the environmental conditions causing the bare patches.

Lower levels of soil K, Mg and moisture were associated with the bare patches in which no grass would grow. However it was difficult to determine if these were causal factors. Deficiencies of K and Mg in the soil can limit vegetation growth (Munshower, 1994). However, the Mg levels in the bare areas and the established stands were within the normal ammonium acetate extractable range for soils, of 40 – 500 mg Kg^{-1} (Grimshaw *et al* 1989). Magnesium although a macronutrient is also only needed in relatively small quantities by plants and, therefore, it is not usually in short supply (Bradshaw & Chadwick, 1980). The lack of any significant differences in plant biomass in the bioassay was also a clear indication that the variability in K and Mg within the soil was unlikely to be the primary cause of the bare patches. In terms of soil moisture, it is difficult to determine if the low soil moisture in the bare areas was a cause or an effect of no grass cover. There was no apparent physical difference in the soil structure, as shown by the stone and clay contents. Therefore, the lower moisture levels in the bare areas were most likely due to increased evaporation from the soil caused by the lack of protection from a vegetation canopy and the higher surface temperature. Thus a lack of soil moisture in areas of the landfill was unlikely to be the cause of patchy grass growth.

High levels of soil Ca and Zn were associated with the bare areas on the landfill, however, again these results do not necessarily show a causal relationship. There is little concern with regards to soil calcium deficiency or excess unless soil pH extremes are apparent (Munshower, 1994). In this investigation the soil pH was not extreme and ranged from, 7.4 - 8.1, therefore, it was unlikely that the Ca levels were directly responsible for the lack of

grass growth. However, elevated Ca levels can influence the availability of essential trace metals, especially Mn and Fe, thus possibly resulting in plant deficiencies (Grimshaw *et al*, 1989). High levels of soil Ca are also generally associated with leachate contamination and are one of the pollutant ions mainly responsible for increased soil salinity (Hernandez *et al* 1999). This may explain the relatively high soil conductivity values recorded throughout the study area.

High soil conductivity as a result of leachate contamination has been shown as the cause of poor vegetation growth on some landfills (Hernandez *et al* 1999; Lan & Wong, 1994; Wong *et al* 1992). However, the contamination of soil with leachate can also be beneficial for plant growth as it can provide much needed moisture and nutrients (Cureton *et al* 1991; Gordon *et al* 1989). Although the mean soil conductivity in this investigation was 5.5 mS cm^{-1} which is above the recommended level, of 2 mS cm^{-1}, for non-tolerant vegetation growth (Bradshaw & Chadwick, 1980; Gilman *et al* 1985; Moffat & Bending 1992), there was no apparent relationship between soil conductivity and the lack of grass growth. The dominant species on the site, *Cynodon dactylon*, has been reported to be leachate tolerant and is commonly used for the reclamation of landfills (Menser *et al* 1979, 1983) and Tong & Wong 1984, showed that *Cynodon dactylon* seed germination was improved by low concentrations of leachate irrigation. Therefore, the natural colonisers of the site appear to be tolerant of leachate contaminated soils and leachate was unlikely to be the cause of the bare areas. Again, if high levels of soil Ca or conductivity were responsible for the bare patches a significant difference in plant biomass in the bioassay would have been expected.

In terms of soil Zn, toxicity is only usually found in soils with a pH below 5.5 (Pais & Jones, 1997). The lowest soil pH measured in this experiment was 7.4.

Diethylenetriaminepentaacetic acid (DTPA) extracted soil zinc reveals a phytotoxic response between 50 and 125 mg kg^{1} (Munshower, 1994). Although, a different extracting solution was used in this investigation, the highest zinc level measured was 27mg kg^{-1} which was considerably below the levels reported to be phytotoxic. Therefore the relationship seen between no grass growth and soil Zn levels was also unlikely to be the key reason for the bare patches.

The data showed an unclear relationship between grass growth and the soil Mn and P levels. However, considering soil P toxicity in the natural environment is unknown and low levels of P are usually the limiting factor (Munshower, 1994), it is unlikely that the high levels of P in the bare areas can be responsible for the lack of grass growth. Leachate contamination of the soil can result in increased Mn concentrations (Lan & Wong, 1994; Winant *et al* 1981), and is often associated with poor vegetation growth (Lan & Wong, 1994; Winant *et al*, 1981, Wong & Yu, 1989). However, Wong and Yu, (1989) found Mn concentration to have a significant negative correlation with forb growth but not grasses, suggesting a possible greater tolerance of grasses. The normal soil range for (ammonium acetate) extractable Mn concentrations is 5 - 500 mg kg^{-1} (Grimshaw *et al* 1989). The established stands of grass in patches 1 and 2 (Table 2.3) had significantly higher Mn concentrations by comparison to the bare areas, however, the concentrations were within the normal soil range (Grimshaw *et al* 1989). Manganese is usually only toxic when the soil pH is low (<5.5) or under strong reducing conditions such as that found in anaerobic soils (Munshower, 1994; Pais & Jones, 1997; Winant *et al* 1981). In this investigation the lowest pH recorded was 7.4 and lowest oxygen level was 8.5%. Therefore, these soils did not have a low pH and were not anaerobic, thus, Mn was unlikely to be toxic. Wong & Yu (1989) found significantly higher extractable Mn concentrations on the Gin Drinker's Bay Landfill,

Hong Kong, by comparison to an off-site control area which had similar soil total Mn levels. The results found by Wong & Yu (1989) and the variation in Mn in relation to the bare areas on the landfill suggest the need for further investigation into this aspect of landfill soil chemistry. It is difficult to get an accurate extractable Mn concentration as the *in situ* redox potential of the soil is difficult to maintain once soils are sampled, thus influencing the availability of Mn.

There was evidence in the correlation analysis to suggest that soil temperature, pH and elevated CO_2 may be responsible for the lack of grass growth in the bare patches. However, the higher soil temperature associated with the bare patches is more likely to be a result of the lack of vegetation, as with moisture, than a cause. Especially considering that the soil structure did not appear to vary significantly within the study site. The pH range found in this study was within the normal range of 4.5 - 8 recommended by McKendry, (1996) for soils used in landfill restoration. Therefore, pH was also unlikely to result in the total lack of grass growth in certain areas and a significant effect on plant growth would have been apparent in the bioassay. The remaining variable that had an apparent association with the bare areas in the study area was elevated soil CO_2. Although the soil gases in the no grass areas and the established stands were both in excess of what would be expected for healthy soils, the correlation analysis suggested a possible relationship between higher soil CO_2 and the lack of grass growth.

From the results of the bioassay and the discussion of the soil chemical data above, landfill gas infiltration into the soil appeared to be the most likely variable responsible for poor grass growth. The concentrations of carbon dioxide ranged from 0 - 39 %, with the lower figures being associated with higher grass biomass (Figure 2.2). The normal range of carbon

dioxide concentrations in the soil atmosphere is 0.1% - 5% (Geisler, 1963; Gendebien *et al* 1992), therefore, the areas on the landfill with carbon dioxide concentrations in the upper part of the range measured were probably exposed to considerable landfill gas infiltration.

Similar research on the bare patches found on landfills has been conducted by a number of other workers (Chan *et al* 1991; Lan & Wong 1994; Wong & Yu, 1989; Wong *et al* 1992; Wong, 1988). The concentrations of all the three gases (methane, carbon dioxide and oxygen) were not always measured, therefore, it is difficult to conclude which gas influenced vegetation the most. However, generally the lower the vegetation cover and plant survival the greater the reported methane and carbon dioxide levels and lower the oxygen levels. As in this investigation, Wong & Yu, (1989) found no significant correlation between the vegetation performance and methane concentrations. Methane does not appear to exert any direct effect upon vegetation, but does reduce the amount of oxygen in the soil by displacement (Chan *et al* 1991, Ettala *et al* 1988, Flower *et al* 1981). However, in this investigation the oxygen levels ranged between 9% - 18% and only when oxygen levels are below 10% are plants usually affected (Flower *et al* 1981). Therefore, it was unlikely that the oxygen levels in the bare areas were responsible for the lack of grass growth. Unlike, methane and oxygen, the relationship between poor grass growth and carbon dioxide was more likely. A significant negative correlation between carbon dioxide and vegetation cover was also found by Chan *et al* (1991) and Wong & Yu, (1989) strengthening the conclusion that the levels of carbon dioxide in the bare areas on the Bisasar road landfill was probably a key variable limiting grass growth.

It is interesting to note that Chan *et al* (1991) measured 82% vegetation cover in an area with a mean carbon dioxide concentration of 17.6 % and mean oxygen concentration of

9.7%. However, on the Bisasar Road landfill, the totally bare areas of the site had lower soil carbon dioxide concentrations and higher soil oxygen conditions. Wong *et al* (1992) measured very similar carbon dioxide (15.1%) and oxygen (12.7%) concentrations, as found on the bare areas in this investigation, in an area of the Gin Drinkers' Bay Landfill with a 33% vegetation cover. A possible explanation for the presence of vegetation in these high gas areas may be attributed to species tolerance. Although, not discussed by Chan *et al* (1991) or Wong *et al* (1992), their results showed the grass *Panicum repens* as the most predominant species on their site, accounting for the majority of the cover measured in the high gas areas. *Panicum repens*, appeared to be a relatively more tolerant species to landfill gas than other species on the Gin Drinkers' Bay Landfill and possibly more tolerant than the species found on the Bisasar Road landfill. It must also be pointed out that the gas measurements made on the Bisasar Road landfill did not account for any temporal variation in gas concentrations that may occur. Therefore higher peak levels of soil carbon dioxide and methane and lower oxygen levels than that measured here, could possibly occur in the bare patches.

Cynodon dactylon, which was the predominant species on the Bisasar Road Landfill, was one of the relatively less common species found by Chan *et al* (1991) and Wong *et al* (1992) and it had a very low cover in the high gas areas. This corresponded with the correlation analysis for the individual species biomass in this investigation (Table 2.5) which showed, although at a level of significance $p<0.1$, a negative correlation between *Cynodon dactylon* biomass and methane and carbon dioxide levels. This suggested that although, *Cynodon dactylon* is tolerant to leachate contaminated soils (Bradshaw & Chadwick, 1980; Menser *et al* 1979, 1983; Tong & Wong 1984), it was sensitive to carbon dioxide and possibly methane levels in the soil.

Although, not discussed by Lan & Wong, (1994) and Wong *et al* (1992), their results showed that *Paspalum sp.* was found mainly in the high gas areas in comparison to lower gas areas. On the Bisasar Road Landfill, *Paspalum paspalodes* was predominately in the border areas of the bare patches, also showing a possible tolerance of the species to landfill gas. However, *Paspalum paspalodes* usually colonises moist areas (Gibbs Russell *et al* 1990) and will probably perform best when conditions are moist, as indicated by the positive correlation between moisture and biomass of this species (Table 2.5). *Sporobolus africanus* has not been found in any other investigations on landfills but the colonisation of the border of the bare areas would suggest relatively higher tolerance of this species to high carbon dioxide concentrations.

Elevated carbon dioxide levels in the soil probably presents the greatest factor limiting grass growth on the landfill. Gas extraction is an expensive and not always successful solution, therefore, the selection of species more tolerant to the conditions is probably a worthwhile solution (Flower *et al* 1981). *Cynodon dactylon* is a good species for revegetation of landfills (Menser *et al* 1983; 1979), however, the possible greater sensitivity to elevated soil carbon dioxide and methane and reduced oxygen levels by comparison to other grass species suggests that other more suitable species may be available.

The use of the *Panicum repens* which appears to colonise areas of similar and higher soil atmosphere gas concentrations, on other landfills, may be a potential solution. *Panicum repens* has a broad distribution in southern Africa and is often found in wet sandy soils, sometimes adjacent to either a fresh or brackish water sources. The species is good for erosion control and is often planted around dams in Zimbabwe (Gibbs Russell *et al* 1990). The results of this investigation indicate that *Sporobolus africanus* and *Paspalum*

paspalodes are also promising species. However, a better understanding of the mechanisms by which landfill gas infiltration limits grass colonisation and growth needs to be attained, thus, facilitating the screening of grass species and possible treatment of the site to improve the success of landfill revegetation.

CHAPTER 3: TREE GROWTH AND SURVIVAL: A PRELIMINARY FIELD INVESTIGATION

3.1 INTRODUCTION

In October 1995 Durban Solid Waste decided to plant trees on the main stability berm of the Bisasar Road landfill, in order to create a rising "green wall" as the landfill site developed. The stability berm already had a grass layer but had extensive erosion. Trees were introduced so as to provide more stability and improve the aesthetics of the site. It was noted by Durban Solid Waste that the trees planted were growing very slowly and a large number had died. This is a commonly found problem with landfill revegetation, especially when trees are used (Chan *et al* 1991; Lan & Wong, 1994; Dobson & Moffat, 1994). However, the use of species that are tolerant to the conditions on landfills can improve the success of revegetation (Flower *et al* 1981; Robinson *et al* 1992).

Although the trees on the stability berm of the Bisasar Road landfill were not planted for research purposes they held the only available information, to our knowledge, regarding South African indigenous tree growth and survival in a landfill environment. The investigation into the health of the different trees species planted and the environmental conditions on the stability berm of the landfill site would provide important information regarding the types and extent of the challenges presented to trees and how they respond.

The results of this preliminary investigation would then allow for further investigations to be more focused on the environmental variables which present the greatest problem and the development of an experimental screening procedure for tree species selection with regard to possible greater tolerance to these conditions.

3.2 MATERIALS AND METHODS

3.2.1 Site description

The main stability berm is situated on the northern side of the landfill site at the bottom of the valley (Figure 1.2). It stretches across the base of the valley and rises to a height of 24 m with a gradient of 1 : 2 and is approximately 230 m wide (Figure 1.2). This stability berm forms the first terrace consisting of the wastes that were first deposited when the site opened in 1980. The front face of the berm is made up of building rubble, rocks and carbonaceous shale covered with a thin layer of various soils. The front face of the berm had been planted with *Cynodon dactylon* and several bands of *Vetiveria zizanoides* (Vetiver grass) to stabilise the slope.

3.2.2 Investigation of the trees planted on the stability berm

In October 1995, an unequal number of twenty different indigenous tree species, approximately 1.5m in height, were planted on the slope of the berm, totalling 210 trees. There was no pre-treatment of the soil on the berm and the trees were planted, at an even distribution across the berm, with only the soil from their potting bags surrounding their roots. The trees received no aftercare, such as watering or weeding.

Surface run-off of rain from the completed section of the landfill above the berm, during the period between October 1995 and February 1996, resulted in extensive erosion of the central section of the stability berm. An unknown number of trees were washed away and destroyed by earth moving machinery used to repair the erosion damage.

A survey of the trees on the berm was conducted in May 1996 so as to determine the actual

numbers of each of the species remaining. The different tree species were identified and the relative position of each of the trees was recorded on an aerial photograph of the stability berm with the aid of an overlaid grid system. Stem diameters were measured 5cm from the ground with digital caliper and the tree height was measured with a steel tape from the ground to the highest shoot. The stem diameter and tree heights were measured for comparison to further measurements to be taken later in the year. All the trees were tagged and their condition was recorded on the 6 May and the 1 August 1996 according to a set of health categories shown in Table 3.1. Although these categories (Table 3.1) were subjective, the classification of the health of the trees on each occasion were completed by the author during a single day so as to reduce possible bias in the results

Table 3.1: Tree health categories based on the general appearance of the trees

Category	Description
1	Very healthy: Full set of leaves with the majority of the leaves not showing any discoloration or chlorosis. Overall good condition with signs of new growth.
2	Healthy: Full set of leaves, however majority showed some signs of discoloration and / or chlorosis. New shoots were present.
3	Poor health: Less than 30% loss of leaves. Leaves remaining maybe discoloured but with majority of leaf area still green. The stems and branches were still flexible and not showing signs of drying out. New shoots were present.
4	Unhealthy: Greater than 40% loss of leaves. Leaves were brown or browning with very little green remaining. Sections of the tree were dead (as described in category 5). No new shoots present.
5	Dead: No leaves, the remaining stem and branches were dry and brittle, no moisture in any of the plant material remaining.

3.2.3 Environmental variables measured on the stability berm

Measurements of the environmental variables were made so as to characterise the general conditions to which the trees were exposed to on the main stability berm. Environmental conditions on landfill sites can have large spatial variation, especially landfill gas (Dobson & Moffat, 1994; Wong *et* al, 1992), therefore, the environmental conditions surrounding each individual tree were measured. The following environmental variables were measured in the soil surrounding the trees: methane and carbon dioxide concentrations in the root zone; soil pH; soil stone content; and % soil moisture.

Landfill gas in the root zone of the trees was of the greatest interest. Therefore, a probe for sampling landfill gas in the root zone needed to be developed. The length of the probe was determined by the depth of the root zone. Considering that adult trees seldom have roots deeper than 1.5m (Dobson & Moffat, 1994), and the trees in question were not adult but only 1.5m in height (whips), the root zone was assumed to be less than 1m below the soil surface. This was confirmed by the excavation of several trees of different species on the stability berm, which were found to have their main root mass not much deeper than 0.45m. The design of a probe was based on that outlined by others (Barry, 1987; Chan *et al*, 1991; Lan & Wong, 1994; Lombard and Associates, 1994; Wong *et al*, 1992). The following design for the probe was utilised: 1m lengths of 24mm outer, 16mm inner diameter plastic water pipe was used. The bottom 35cm was drilled with 5mm holes, 5cm apart to allow for the gas in the root zone to migrate into the probe (Figure 3.1). The top of the pipe was capped with a plastic airtight stopper to prevent dilution of the landfill gas by direct atmospheric exposure. The probes were positioned randomly within a 0.5m radius of the stem of each tree, but in such a way as not to damage the tree, and were inserted to a depth of 50cm below the soil surface. The probes were all inserted to the same depth as gas

concentrations are also found to vary with soil depth (Dobson & Moffat, 1994).

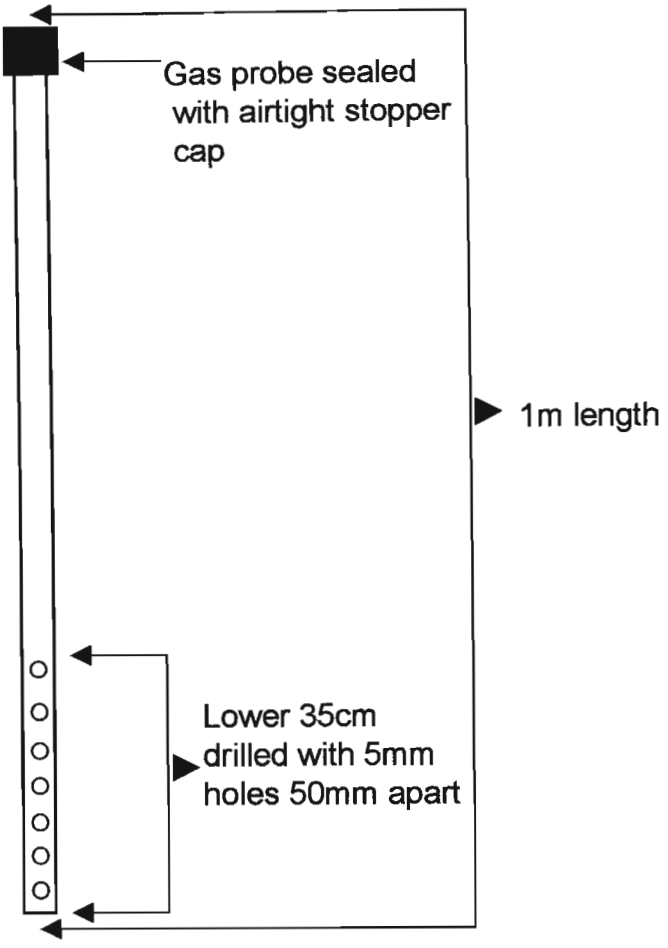


Figure 3.1: Gas probe made of 24mm plastic tube, used for sampling landfill gas within the root zone

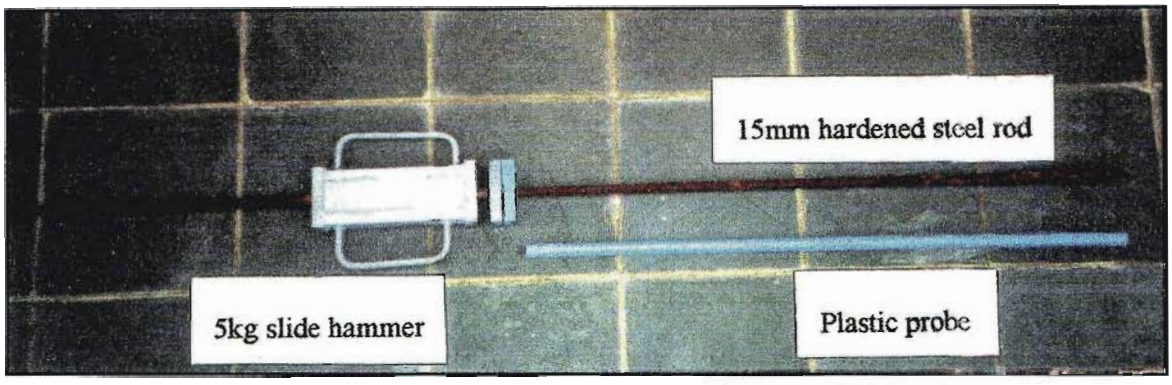


Figure 3.2: A sharpened 15mm diameter hardened steel rod onto which the probe could slide was fitted with a 5kg slide hammer, this was used to insert the probes into the hole created by the dynamic cone penetrometer (D.C.P).

A device for inserting the probe into the ground needed to be constructed. As suggested by Barry (1987), a steel rod was driven into the ground using a slide hammer to make the hole for the probe to be inserted. However, upon removal of the steel rod the hole became clogged with stones and loose soil making it impossible to insert the probes. So as to overcome this problem a sharpened 15mm diameter hardened steel rod, fitted with a 5kg slide hammer, onto which the plastic probe could slide was constructed (Figure 3.2). Using a dynamic cone penetrometer (D.C.P.) with a 50mm diameter head, a hole was driven into the ground. The D.C.P. was then removed and with the support of the hardened steel rod the plastic probe was driven into the hole. Once the steel rod was removed the probe was sealed with a plastic cap and the surrounding loose soil was packed tightly against the sides of the probe to prevent gas escaping. Gas samples were taken from the probes (which remained in the ground) at intervals for the duration of the investigation.

Although methane is less dense than air, when it is mixed with other landfill gases and diluted it may not be very buoyant unless there is a considerable temperature or pressure difference (Barry, 1987). It is for this reason that an aspirator must be used for the removal of a sample of gas from the probes. Gas samples taken from the probes can be analysed using a gas chromatograph but this is an expensive procedure, especially when considering the large number of probes and replicated measurements required for this investigation. The availability and expense of a portable carbon dioxide and oxygen meter also presented a problem. However, for this investigation a portable methane meter (G624p Exotector) with a built in aspirator was available. The methane meter determined the percentage methane by the thermal conductance characteristics of the gas sample taken. Methane is not directly toxic to plants, but methane concentrations can be regarded as an indirect indicator of concentrations of more important gases i.e. high carbon dioxide and low

oxygen (Chan *et al*, 1991). For comparison with the methane concentrations measured in the root zone, a portable carbon dioxide meter (Draeger Multiwarn, Infrared CO_2 0-100%, No. 6807940) was used for CO_2 measurements, however, it was only available for a limited period.

Methane was measured either in the early mornings, at midday or in the late afternoon on five separate days. Gas measurements were taken once a day so as to ensure that gas concentrations in the probe had time to equilibrate with the root zone soil atmosphere. Variations in temperature, rainfall and barometric pressure tend to influence landfill gas emissions (Chan *et al*, 1991; Dobson & Moffat, 1994; Lombard & Associates, 1994). Therefore, temperature and barometric pressure were recorded with each set of gas measurements made. Carbon dioxide was measured on only two separate occasions due to the availability of equipment. The mean value for the gas concentration from each probe was compared with data from all the probes in order to locate areas with high landfill gas on the stability berm. The gas concentration measurements were also compared to the relative health of the trees.

Soil samples were collected using a soil auger from the top 15cm to 20cm of soil. Two auger samples were taken randomly within a 0.5m radius of each tree. The two soil samples were immediately sealed into a polythene bag and mixed. The samples were transported back to the laboratory where pH, stone content, and moisture content were determined.

The pH of the fresh soil samples was measured using a pH electrode (Hach Model 43800) with a 1:1 ratio of soil to distilled water (Grimshaw, 1989). Any large stones were removed

from the samples for pH analysis so as to prevent damage to the electrode. pH measurements were replicated three times for each soil sample. For the analysis of stone content the soil samples were air dried at room temperature (Grimshaw, 1989) and the soil aggregates were gently broken up with a mortar and pestle. The sample was separated with a 2mm sieve dividing the soil from the 'stone' (>2mm fraction). The weight of stones was then expressed as a percentage of the total weight of the soil sample that was sieved. The moisture content of the fresh soil samples were measured as described by Grimshaw (1989). 10-20 g fresh soil samples, with large stones and roots removed, were weighed in dry evaporation basins. These samples were placed in an air circulation oven at 105°C until they reached a constant weight. They were then cooled in a desiccator and the percentage fresh moisture was calculated from the loss in weight. Each sample was replicated three times.

Statistical analysis of the data collected was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data were analysed using an analysis of variance. If there was a significant difference ($p<0.05$) in data with more than two sample variables then Scheffe multiple range test was performed by constructing intervals for pair-wise differences of means to determine which differences were significant ($p<0.05$).

3.3 RESULTS

3.3.1 The trees on the stability berm

A total of 210 trees comprising of twenty different species were listed to have been planted on the main stability berm (Table 3.2). All of the trees that were planted were staked into the ground (D. Dorkin, 1996 *pers comm*), therefore, although the trees may have died the

stakes would still remain indicating the position of the tree. However, the survey completed in May 1996 revealed that only 110 trees alive or dead were present (Table 3.2). An average of only 47 % of the total individuals of each species initially planted was actually found with some species not being found at all.

Table 3.2: A list of the tree species planted in October 1995 on the main stability berm and the numbers of these trees found in the survey carried out in May 1996.

Species	No. supplied	No. recorded in survey
Acacia sieberiana	12	7
Acacia xanthophloea	4	4
Celtis africana	13	6
Combretum erythrophyllum	20	10
Cussonia spicata	8	0
Dais cotinifolia	10	0
Dombeya rotundifolia	8	3
Erythrina lysistemon	11	8
Harpephyllum caffrum	12	3
Heteropyxis natalensis	6	4
Hibiscus tiliaceus	4	3
Peltophorum africanum	4	2
Rhus lancea	17	14
Schotia latifolia	8	5
Schefflera umbellifera	2	0
Strelitzia nicolai	20	2
Syzygium cordatum	30	28
Tabernaemontana ventricosa	11	0
Trema orientalis	6	4
Ziziphus mucronata	4	0
Support stakes without trees [1]	--	7
TOTAL	210	110

[1] All the trees planted were staked into the ground, therefore, although trees may have died the stakes could remain, indicating the position of the tree.

Since October 1995 considerable erosion of the central portion of the stability berm had taken place and earth works were completed so as to repair this damage. This operation

and the erosion probably accounted for a large number of trees being destroyed.

Due to the low numbers of each tree species the health category system (Table 3.1) was simplified, as explained in Table 3.3. The condition of each species was expressed as a proportion of healthy trees of that species found on the berm (Table 3.3).

Table 3.3: Proportion of the trees of each species found on the stability berm which were healthy in May 1996[1]

Species	Proportion healthy [1]	No. of trees of each species
Strelitzia nicolai	1	2
Harpephyllum caffrum	1	3
Acacia xanthophloea	1	4
Rhus lancea	0.68	14
Hibiscus tiliaceus	0.67	3
Combretum erythrophyllum	0.55	10
Acacia sieberiana	0.5	7
Peltophorum africanum	0.5	2
Schotia latifolia	0.5	5
Celtis africana	0.42	6
Heteropyxis natalensis	0.38	4
Dombeya rotundifolia	0.33	3
Syzygium cordatum	0.29	28
Trema orientalis	0.25	4
Erythrina lysistemon	0	8

[1]Proportion of healthy trees calculated using a simplified version of the health ranking system (Table 3.1). Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy. Trees that were ranked as 3 were divided and 0.5 was added to the healthy and unhealthy groups. Thus, proportion healthy = [(No. of trees ranked 1 & 2)+(No. of trees ranked 3 x 0.5)] ÷ (Total number of trees of the species)

All the *Strelitzia nicolai, Harpephyllum caffrum* and *Acacia xanthophloea* trees on the stability berm were 'healthy'. *Rhus lancea, Hibiscus tiliaceus* and *Combretum erythrophyllum* had predominantly 'healthy' trees growing on the berm. *Peltophorum*

africanum, *Schotia latifolia*, and *Acacia sieberiana* had the same proportion of 'healthy' and 'unhealthy' trees growing on the berm. However, *Trema orientalis*, *Syzygium cordatum*, *Dombeya rotundifolia*, *Heteropyxis natalensis* and *Celtis africana* had low proportions of healthy trees. No 'healthy' trees of *Erythrina lysistemon* were found on the stability berm in May of 1996. It must be noted that for some species with low numbers of individuals, the proportion of 'healthy' individuals may not accurately represent the species performance under landfill conditions. There is often large spatial variation in the environmental conditions on a landfill, therefore, the smaller the number of trees, the greater the chance that all the trees of one species trees may have only been planted in either, an exceptionally harsh or, a favourable area of the berm.

For further analysis of these data, the number of species was reduced to seven species that had greater than 5 individuals, allowing for a more focused investigation of the individual species in relation to the environmental variables measured. The selection of the species was further reduced to five, that is those species which were ranked predominately 'very healthy' (category 1), namely *Rhus lancea*, *Combretum erythrophyllum*, *Acacia sieberiana*, or 'dead' (category 5), namely *Syzygium cordatum* and *Erythrina lysistemon*. This was done as the number of individuals within each health category for *Celtis africana* and *Schotia latifolia* was too low for meaningful results to be obtained.

The health category measurements made on these five tree species in May were repeated in August (Table 3.4). A comparison of the measurements between May and August showed, that unlike the other four species, *Erythrina lysistemon* had a marked increase in the proportion of healthy trees. In May *Erythrina lysistemon* was classified as unhealthy because of its lack of leaves when this was in fact probably a seasonal effect. By August

Erythrina lysistemon was no longer dormant and began to grow, thus, the August health measurements provided a better representation of the condition of the species. For the other two deciduous species, *Acacia sieberiana* and *Combretum erythrophyllum*, there was no improvement in health between May and August, suggesting that the health of these species was accurately observed and the results were not affected by seasonal changes.

For the non-deciduous species, *Syzygium cordatum* and *Rhus lancea,* there was unlikely to be any seasonal influence, therefore, the proportion of healthy trees probably provided a good representation of health condition of the species. The proportion of healthy trees of *Syzygium cordatum* was much lower in August by comparison to April showing a deterioration in tree health (Table 3.4), whereas *Rhus lancea* had very little change in the proportion of healthy trees. It must be noted that further observations of the trees over a longer period of time, preferably more than one season, would have provided a better indication of the performance of the species.

In order to obtain estimates of species growth rates stem diameter and tree height were recorded in May 1996, by comparison with measurements to be made later in the year. However, due to the unforeseen construction of a rainwater drainage pipe down the centre of the stability berm and a gas reclamation pipeline diagonally across the stability berm in October 1996, 30% of the trees measured in May on the stability berm were destroyed. The number of replicates for each tree species became too low for the growth rate results to have any statistical validity and therefore, this study was abandoned.

Table 3.4: The proportion of healthy[1] trees for the five tree species in May and August 1996 and the most likely reason for the change (health effect: change due to deteriorating health of tree; Seasonal effect: change due to tree emerging from winter dormancy).

Species	May	August	Possible reason for change
Deciduous species*			
Acacia sieberiana (n=7)	0.5	0.36	Health effect
Combretum erythrophyllum (n=10)	0.55	0.2	Health effect
Erythrina lysistemon (n=8)	0	0.63	Seasonal effect
Non deciduous*			
Syzygium cordatum (n=28)	0.29	0.09	Health effect
Rhus lancea (n=14)	0.68	0.64	Little change

[1] Proportion of trees healthy calculated using a simplified version of the health ranking system (Table 3.3). Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy. Trees which were ranked as 3 were divided and 0.5 was added to the healthy and unhealthy groups. Thus, proportion healthy = [(No. of trees ranked 1 & 2)+(No. of trees ranked 3 x 0.5)] ÷ (Total number of trees of the species)

* As described by Palgrave, 1984

3.3.2 Environmental variables

The mean percentage methane in air recorded within the root zone on the stability berm was 13.6 (std error 1.2) with a large range between 0 and 60%. The mean percentage carbon dioxide in air within the root zone was 4.2 (Std error 0.5) with a minimum of zero and a maximum of 22%. The mean carbon dioxide and mean methane measured at each probe had a significant ($p<0.05$; $R^2 = 0.63$) linear relationship, with carbon dioxide increasing with methane concentrations. There was no significant variation ($p>0.05$) in methane concentrations measured in the early morning, midday or late afternoon. There was also no significant ($p>0.05$) variation in methane measured at different barometric pressures and temperatures, however, the range of atmospheric temperature (18°C - 29°C) and pressure (1024 mb - 1039 mb) was relatively small.

The health measurements made in August were used for comparison with the environmental variables measured. However, the health category system (Table 3.2) was again narrowed down from five categories into two: healthy; and unhealthy (i.e. Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy, as shown in Table 3.3. The individual trees ranked as 3 were not divided and 0.5 added to the healthy and unhealthy categories for the species, as done before. The health classification of individuals, in August, with a health rank of 3, was determined by the change in health of the individual tree between May and August. If the health of the individual tree had deteriorated between May and August it was classified as unhealthy and *visa versa* for those individuals put into the healthy category.

The comparison of the health of the trees (all species combined) on the stability berm with the mean methane concentrations measured in the root zone, showed that the trees classified as unhealthy had significantly ($p<0.05$) higher root zone methane concentrations by comparison to the healthy trees. Similarly, the analysis of the root zone methane concentrations for the individual species gave the same conclusion, with a significantly ($p<0.05$) higher methane concentration in the root zone of the unhealthy trees of each species, except for *Erythrina lysistemon* (Figure 3.3). *Erythrina* had no statistically significant ($p>0.05$) difference in methane concentrations between healthy and unhealthy trees. It is important to note that the numbers of individuals within one of the two health categories was often very low, especially for *Acacia sieberiana* and *Syzygium cordatum*, thus, limiting the interpretation of the results (Figure 3.3). However, the results suggest that concentrations of methane in the root zone were related to the health of the trees. Out of the five species, the health of *Erythrina lysistemon* appeared to be the least affected by the methane in the root zone.

Figure 3.3 shows that not all of the species were exposed to the same average methane concentrations. This can be attributed to the large spatial variation in the landfill gas concentrations on the stability berm. It is important to note that the healthy trees of *Acacia sieberiana*, *Syzygium cordatum* and *Combretum erythrophyllum* were found in areas of very low methane (< 2%) concentration. Whilst, the healthy trees of *Rhus lancea* and *Erythrina lysistemon* were found in areas of considerably higher methane, 9% and 20 % respectively. This suggests that *Erythrina lysistemon* and *Rhus lancea* were less susceptible to higher methane concentrations in the root zone by comparison to the other three species. However, the low numbers of individual trees for each species in most of the two health categories indicate that these conclusions should be treated with caution.

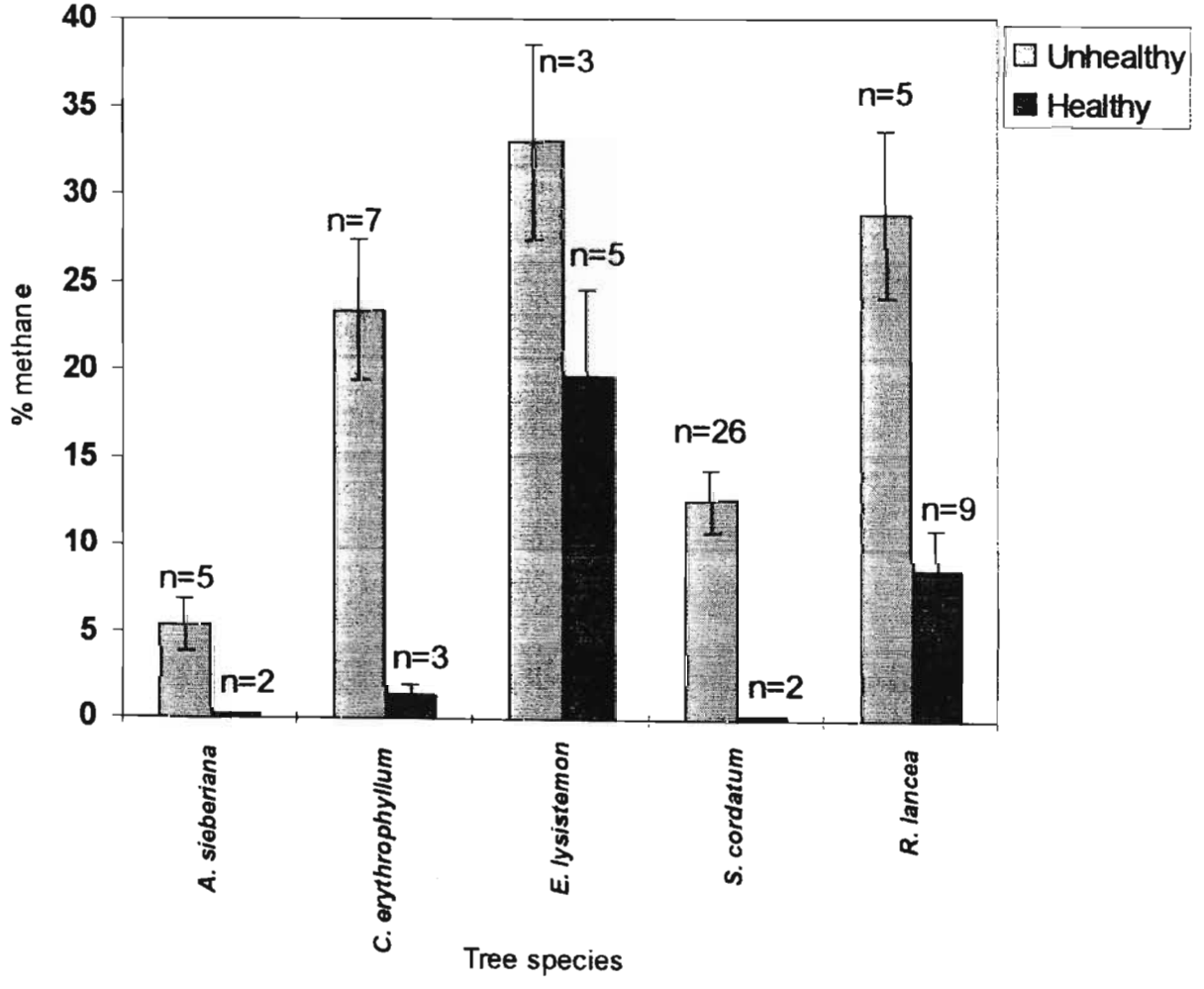


Figure 3.3: The relationship between methane in the root zone and species health in August 1996. Results are mean values with standard errors.

The analysis of the soil on the stability berm showed that the mean pH was 6.42 (Std error 0.05) with a minimum value of 4.8 and a maximum of 8.0. There was no significant ($p>0.05$) difference in soil pH between the healthy and unhealthy trees on the stability berm. The same was the case for the analysis of the soil pH for the individual species, except for *Acacia sieberiana*. The unhealthy trees of *Acacia sieberiana* had a significantly ($p<0.05$) higher soil pH when compared to the healthy trees of the same species (Figure 3.4). However, the pH was not significantly ($p>0.05$) higher than the pH conditions that the other four species were exposed to. The significant difference in *Acacia sieberiana* soil pH may suggest that species preferred lower soil pH. The results generally suggested that soil pH was probably not one of the main variables influencing the health of the trees.

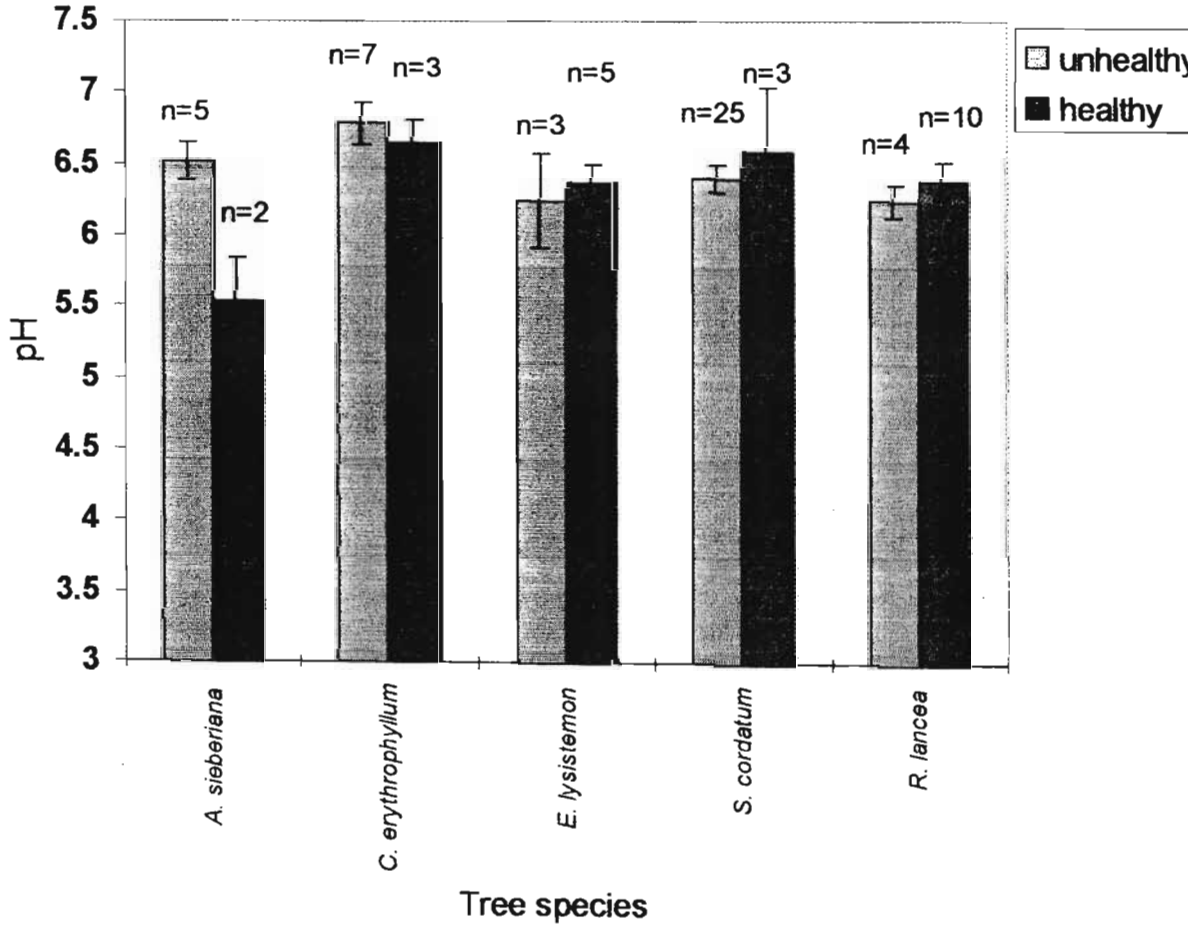


Figure 3.4: The relationship between soil pH and species health in August 1996. Results are mean values with standard errors.

The mean percentage soil stone content on the stability berm was 39.95% (Std error 1.18%). The highest stone content measured was 77.1% and the lowest 19.1%. No significant difference ($p<0.05$) was found between the soil stone content for healthy and unhealthy trees for the analysis of all the tree species or the individual species (Figure 3.5). This showed that although the stone content of the soil was high, it did not appear to be a primary cause for the difference in health of the trees.

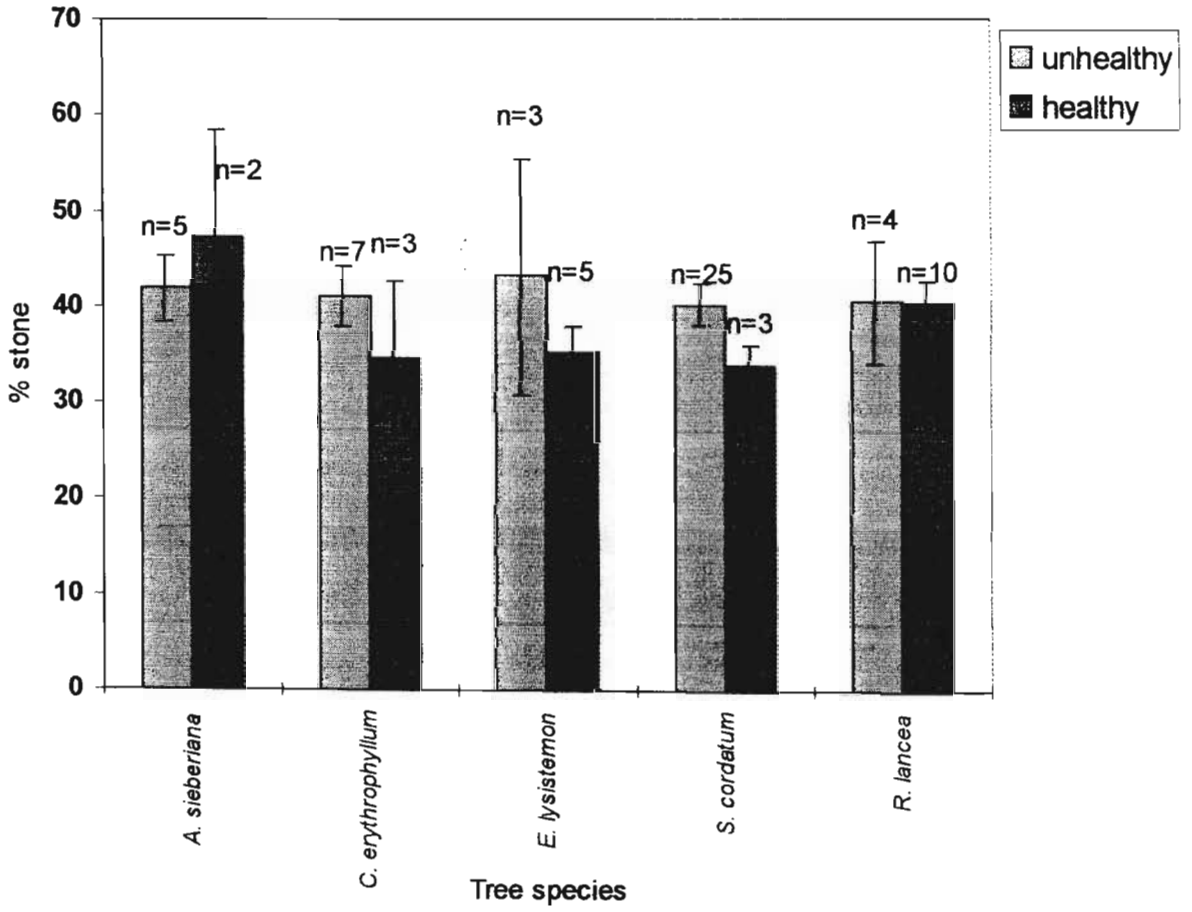


Figure 3.5: The relationship between % stone and the species health in August 1996. Results are mean values with standard errors.

The mean percentage soil moisture on the stability berm was 14.75 % (Std error 0.19) with a minimum of 4.5% and a maximum of 23.4%. Figure 3.6 shows the different soil moisture contents found in relation to healthy and unhealthy trees. *Acacia sieberiana*, *Combretum*

erythrophyllum and *Rhus lancea* had no significant ($p<0.05$) difference in soil moisture between healthy and unhealthy plants. However, the unhealthy trees of *Erythrina lysistemon* and *Syzygium cordatum* were exposed to a significantly ($p<0.05$) lower soil moisture content by comparison to the healthy trees. This could possibly suggest that the poor health of some individuals of *Erythrina lysistemon* and *Syzygium cordatum* may be due to soil moisture conditions.

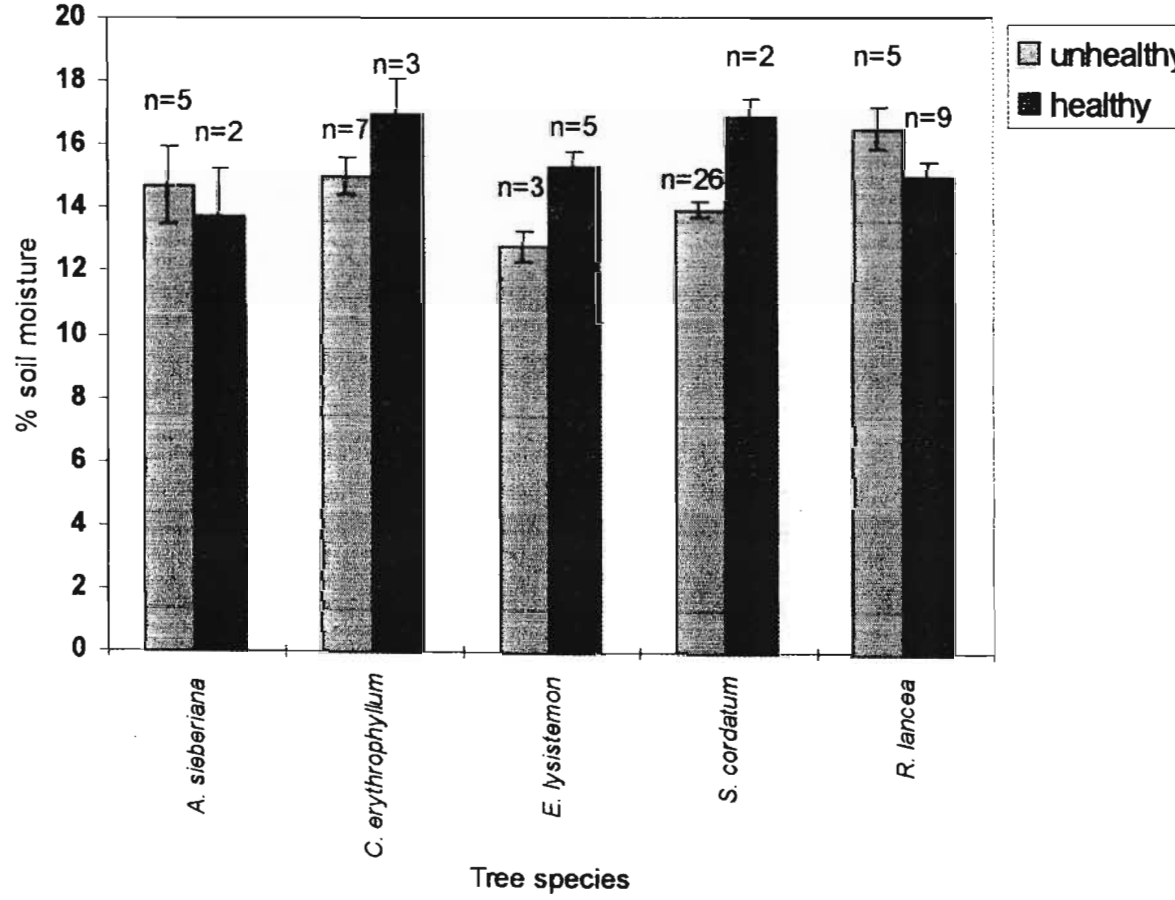


Figure 3.6: Relationship between soil moisture and species health in August 1996. Results are mean values with standard errors.

3.4 DISCUSSION

Taking into account that the landfill site is still fully operational and only a section was being revegetated, the major cause of tree mortality was due to disturbance by earth moving machinery. This point illustrates that over and above consideration for the harsh environmental conditions on landfill sites it is important to have careful planning and management of areas that are being revegetated in order for successful vegetation establishment to be achieved.

Unfortunately, for many of the species planted the replication was too small for conclusions to be made, therefore, only the performance of the following species could be assessed in greater detail: *Acacia sieberiana, Combretum erythrophyllum, Erythrina lysistemon, Syzygium cordatum* and *Rhus lancea*. However, although the numbers of individuals limited the interpretation of the data, *Strelitzia nicolai, Harpephyllum caffrum, Acacia xanthophloea* and *Hibiscus tiliaceus* appeared to be the relatively more healthy species out of those which had less than five individuals (Table 3.3).

Seasonal variation in deciduous trees effected the health ranking system (Table 3.4). *Erythrina lysistemon* was a good example of how a deciduous species which previously (May 1996) appeared 'unhealthy' became considerably more 'healthy' later in the year (August 1996). This emphasises the need for a less subjective and more absolute measure of plant health. It also highlighted the need for long term observation through all of the seasons, especially for deciduous species, in order to get a more accurate interpretation of the species performance. Unfortunately many of the trees were destroyed in October making the longer term monitoring of the trees on the stability berm impossible.

In terms of the effect of the environmental variables measured on the health of the trees, it would appear that soil pH, stone content and % moisture had little influence on the trees. Very little variation in pH throughout the area of investigation was measured. The soil pH on the stability berm was within the normal range of pH 4 – 8 for landfill restoration (McKendry, 1996; Moffat & Bending, 1992).

The mean stone content of the soil, of 40% (±1.2%), greatly exceeded the soil specification standards for landfill restoration of <10% (2-50mm) by dry weight (McKendry, 1996). However, in the comparative study here, the stone content of the soil did not affect the health of the trees. Stone content probably only represents an important factor when within a small size range (2-25mm), where it can prevent seed germination and root development (Mc Kendry, 1996). Although the stability berm cover material consists of a high percentage of stones within a small size range (2-25mm), the trees were several years old and had developed roots when planted, and so would not be as easily affected.

Syzygium cordatum and *Erythrina lysistemon* were the only two species that showed any significant difference between the two health categories for soil moisture (Figure 3.6). *Syzygium cordatum* is naturally always near water and often forms stands in pure swamp forest (Palgrave, 1984), therefore it is very likely to be sensitive to moisture conditions and will find low moisture levels challenging. *Erythrina lysistemon* is found in a much wider range of habitats from dry woodland to coastal dunes but usually in high rainfall areas (Palgrave, 1984). However, the unhealthy specimens of *Erythrina lysistemon* were exposed to lower soil moisture conditions in comparison to the other tree species (Figure 3.6), possibly providing an explanation for the significant difference in health for soil moisture. The general pattern of lower soil moisture for unhealthy plants (Figure 3.6), although not

significant, may indicate that moisture could present a problem to tree health during the months with lower rainfall.

High methane concentrations were closely associated with the poor health of the trees on the stability berm, as found in many other investigations (Chan *et al* 1991; Flower *et al*, 1981; Flower *et al*, 1977; Spreull & Cullum, 1987). This suggested that landfill gas infiltration into the soil atmosphere in the root zone was the key environmental variable measured influencing tree health. Methane is not directly toxic but is a good indicator of the presence of other toxic landfill gas components (Chan *et al* 1991). The high methane concentrations usually indicates anaerobic soil conditions and the possible presence of toxic gases such as carbon dioxide, ethylene and hydrogen sulphide which are often responsible for poor tree health (Leone *et al* 1977, Dobson & Moffat, 1994).

Soil carbon dioxide levels increased in a linear manner with soil methane concentrations confirming the findings of Chan *et al* (1991) and Lan and Wong, (1994). This indicated that the high carbon dioxide levels were also associated with the poor health of the trees. Carbon dioxide is an important component of landfill gas as it is toxic to plants in high concentrations (Arthur, *et al* 1981; Barry *et al* 1987; Chan *et al* 1991; Flower *et al* 1981; Leone *et al* 1977). The twenty two percent carbon dioxide concentration measured in high gas areas of the stability berm was higher than the range of 15-20%, which is lethal to most plants (Chan *et al* 1991; Chang & Loomis, 1945). Therefore, carbon dioxide levels were likely to be responsible for a large proportion of the trees poor health, however, low oxygen and trace gases such as ethylene and hydrogen sulphide may also have contributed to poor tree health.

The entire stability berm was characterised by patches of high and low methane and carbon dioxide concentrations, indicating a large spatial variation in landfill gas, commonly found on landfills (Wong *et al* 1992). Unlike that found by Lombard and Associates (1994), no variations in landfill gas concentrations in relation to atmospheric conditions were measured, however, the range of atmospheric temperature and pressure causing variations in landfill gas levels were not reported by Lombard and Associates (1994). A possible explanation for the lack of variation in landfill gas concentrations with climatic conditions, in this investigation, may be the small variation in these conditions experienced during the survey period.

The tree species were found to respond differently to the methane concentrations possibly indicating differential tolerance to landfill gases. *Acacia sieberiana* and *Syzygium cordatum* were found to have little or no tolerance with no healthy plants found exposed to methane. *Combretum erythrophyllum* had healthy trees surviving in very low methane concentrations. *Rhus lancea* had healthy trees surviving at a relatively higher mean methane concentration of approximately 9%. However, the species which showed the most tolerance to landfill gas was *Erythrina lysistemon*, which showed no significant difference between healthy and unhealthy species even though it was exposed to the highest methane concentrations of approximately 34% (Figure 3.3).

This investigation provided insight into the problems and challenges associated with revegetation of a landfill. It isolated two key factors associated with tree death, namely human disturbance, which refers to the unforeseen earth moving activity, and landfill gas. Better management and control can remove human disturbance, however, the removal of landfill gas is expensive, and not entirely successful, therefore, the search for tolerant

species is of significance. The species planted on the site provided preliminary data suggesting that there was a range of tolerance within indigenous tree species, which would be worthwhile investigating further.

CHAPTER 4: TREE GROWTH AND SURVIVAL: A FIELD EXPERIMENT

4.1 INTRODUCTION

The benefits of encouraging vegetation growth on operational and complete landfills has been well documented (Dobson & Moffat, 1994; Erickson *et al* 1994; Ettala *et al* 1988; Menser *et al* 1979). Trees have an especially important role, in terms of aesthetics, when reclaiming completed sites for parks, golf courses, and other similar amenities as well as for the screening of operational sites (Dobson & Moffat, 1994; Flower *et al* 1981). However, there are many factors limiting plant growth, especially trees, on landfills (Chan *et al* 1991; Lan & Wong, 1994; Dobson & Moffat, 1994; Ettala *et al* 1988; Flower *et al* 1981; Gill, 1970; Gilman *et al* 1981; Insley & Carnell, 1982; Leone *et al* 1983; Leone *et al* 1977; Moffat & Houston 1991). The amelioration of these factors can be very expensive and often less than completely successful. Therefore, the use of tree species tolerant to landfill conditions, when possible, can be of great benefit for revegetation success (Flower *et al*, 1981; Robinson *et al* 1992). The present study investigated the relative tolerances of indigenous tree species to the landfill environment with a special emphasis on landfill gas.

Using the results from the preliminary investigation (Chapter 3), ten indigenous tree species were selected for a more rigorous and on-site field study. The experimental screening of species in the field prevents the elimination of minor, or unforeseen detrimental environmental conditions. These may individually or in combination limit tree growth and survival. The hoped for outcome being the selection of species that are tolerant to the landfill environment as a whole and not just particular, individual components. In summary, the experiment has an element of a bioassay approach together with the measurement of certain variables to investigate the reasons for any differences in tree

performance. These measured variables included concentrations of gases (methane, carbon dioxide, oxygen) and the temperature of the soil in the root zone, and basic soil physical and chemical characteristics. This provided for some insight into the reasons for poor tree growth and thus a focus for amelioration procedures to overcome the potentially limiting environmental factors, and so facilitate successful tree establishment on landfills.

4.2 MATERIALS AND METHODS

4.2.1 Species selection

Nine tree species from the preliminary investigation were selected using two criteria: firstly, that they were readily available from commercial retailers in numbers greater than 70; and secondly, that they were the most successful in terms of survival in the preliminary investigation on the main stability berm. The majority of the species which survived best on the stability berm tended to be those found naturally growing in potentially waterlogged habitats (Palgrave, 1984; Pooley, 1994). To investigate further this assumption *Barringtonia racemosa,* a commercially available tree species which is characteristically found in swamp forest communities (Palgrave, 1984; Pooley, 1994) was added to the list of species to be screened. Therefore, the following ten experimental species were chosen: *Acacia sieberiana; Acacia xanthophloea; Barringtonia racemosa; Combretum erythrophyllum; Hibiscus tiliaceus; Erythrina lysistemon; Harpephyllum caffrum; Rhus lancea; Strelitzia nicolai* and *Syzygium cordatum.*

4.2.2 Experimental design

Considering that landfill gas in the soil was a key environmental condition related to poor tree health, the presence of landfill gas in the area to be used in the field experiment was

essential. In the preliminary investigation (Chapter 3) the lack of homogeneity of landfill gas concentrations (measured as methane concentrations) in the soil made the assessment of particular species performance in relation to landfill gas concentrations difficult. Therefore, for the field experiment it was important that an area of the landfill which was relatively homogenous in terms of landfill gas concentrations in the soil was used. An area of the landfill was investigated for its suitability for the field experiment (Figure 1.2). This area was temporarily complete and had approximately 30m of waste underneath it, which had been in-filled since 1980, and then covered with approximately 0.5m of waste soil. This area was beyond the effective range of the recently installed gas reclamation wells (Dorkin, D. 1996 *pers comm*), thus ensuring a negligible effect of active gas removal on the concentrations of landfill gas in the soil.

A 50m by 50m section of the area was then selected for its relatively flat topography and homogeneous appearance in terms of soil structure and moisture (Figure 1.2). Within this 50m by 50m section 13 gas samplers, with the same design as those used in the preliminary investigation (Chapter 3), were installed in a grid pattern. Methane concentrations in the soil were measured once a week for three weeks (Table 4.1). Table 4.1 shows that the spatial and temporal variation in methane concentrations during the 3-week period of monitoring was acceptably low. The over-all mean methane concentration for the plot was 52 % which was considerably higher than the mean value of 14% measured on the stability berm in the preliminary investigation. Although the gas concentrations were considerably higher, the plot was regarded as suitable for the field experiment. There was very little variation spatially or temporarily for 10 of the sampling points in the plot. However, three areas of the plot did have lower methane in the soil, as indicated by the measurements from gas samplers 8, 10 and 13 (Table 4.1), indicating a

slight variability in gas emissions within the plot. It was, therefore, decided that a replicated grouped experimental design (see below) would be the best for the planting of the trees, so as to account for this apparent heterogeneity where pockets of lower concentrations may be found.

This 50m by 50m area was completely fenced so as to prevent any accidental damage to trees by vehicles during the field experiment. Within the fenced area two 25m by 25m experimental plots were established. One received 1m of topsoil (the topsoil plot) whilst the second plot received no topsoil and had only the original 0.5m deep waste soil cover material. A control plot was situated off the landfill approximately 1000m away from the experimental plots, in the Randles Road Municipal Nursery (Figure 1.2). The topsoil used in the experiment was loose tipped into position using a back actor excavator. Five gas samplers which were installed on this control plot detected no methane during a monitoring period of 3 weeks (4-18th November 1996). The underlying substrate of the control plot was yellow clay resulting from the extensive weathering of a dolerite intrusion. On top of soil present at the control site a 1m layer of topsoil, from the same well-mixed stockpile as used on the first plot on the landfill (the topsoil plot), was also placed.

In each of the 3 plots the trees were planted in seven replicated groups. Each of the seven groups had one replicate tree of each species, planted randomly at 1.5m centres. The grouped planting of species was regarded as a more satisfactory way of accounting for site and substrate heterogeneity than a strictly random design for the whole plot. In particular, it was possible that there were pockets of higher or lower landfill gas concentrations in the plots on the landfill (Table 4.1).

Table 4.1: Methane concentrations measured at 13 sample points in the soil within a 50m by 50m area of the landfill between the 4th and the 18th of November 1996, in assessment of its suitability for a field experiment

	% methane		
Gas sampler	Week1	Week 2	Week 3
1	65	65	66
2	60	58	60
3	60	60	58
4	55	55	55
5	55	55	55
6	55	55	55
7	55	55	55
8	55	30	15
9	56	56	55
10	32	35	30
11	55	55	55
12	51	65	60
13	35	40	35
Mean % methane (Std. Error)	53 (3)	53 (3)	50 (4)

The trees were obtained in January 1997 from Randles Road Municipal Nursery in 6*l* potting bags. Individual plants of each species were selected so as to ensure they were approximately the same age and size (2 years old). The potting bags were cut off and the tree roots were then slightly loosened and planted with the attached potting soil directly into the ground of each plot. After planting they were provided with water on a daily basis for the first 4 weeks only. Aftercare of the trees involved the regular weeding of the plots to prevent competition from naturally established grasses and forbs.

4.2.3 Tree performance

Stem diameter and height growth, survival, leaf chlorophyll fluorescence, general health appearance, total aboveground biomass, total leaf area and rooting depth were used to determine treatment effects and differential species response.

The stem diameter and the height of the trees was measured when they were first planted (20/1/97), after 7 months (20/8/97) and finally after 14 months (20/4/98). Data was expressed as the growth increment between these dates. Stem diameter was measured 50mm from the base of the stem using electronic digital callipers. To avoid inaccuracy due to the non-symmetrical shape of stems the orientation of the diameter measurement was taken consistently along a north -south axis. The tree height was measured from the base of the stem to the apical shoot using a steel tape.

The general appearance of the individual trees was also monitored as an assessment of the tree health. The trees were observed and put into one of five categories according to their overall appearance which provided a ranking system from 'dead' to 'very healthy', as used for the trees in the preliminary investigation (Table 3.1). Although this system intrinsically was subjective, the health rankings were all completed by the same person so as to help remove bias in the results. The number of trees of each species that were still alive within each treatment at the end of the experiment provided the measurement of survival.

The above ground biomass was calculated by adding the dry mass of the stem and leaves of each of the trees after the 14 month experimental period. Due to the size and number of trees the dry mass of the stem and leaves was calculated from the fresh weight by drying a sample from each species from each of the experimental plots and calculating a fresh

weight to dry weight correction factor. Although the trees were roughly the same age and size when they were originally planted, the final mass of the trees was expressed as a ratio of the original height of the tree (relative biomass) in order to standardise the data. The total leaf area of each tree was calculated by determining the ratio of leaf mass to leaf area of a sample of leaves and then using this value to estimate the total leaf area from the total leaf mass for each tree.

In order to describe the root morphology of the trees on the control and landfill plots a profile wall trench was excavated for each species on each plot (Total n=30). Using a back actor excavator a 1m deep trench was excavated 300mm from the base of the stem of each tree. The profile wall was levelled with a straight edge and the protruding roots were trimmed. A 100cm X 90cm steel grid, divided into 10cm square blocks was placed onto the profile wall and the roots within the <5mm, 5-10mm, >10mm size classes, within each block were recorded. However, in practice there was a very small range in root diameters seen in the profile walls with 99.5% of the roots less than 5mm in diameter. Therefore the size classes were not used and overall root density with depth was assessed.

4.2.4 Soil gases and soil temperature

Seven gas probes, of the same design to those used in the preliminary investigation (Figure 3.1), were inserted into the substrate in each of the three plots, one within each replicated group of trees. Gas samples were monitored on a monthly basis for percentage methane, carbon dioxide and oxygen in air with a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. Thus monitoring the gas concentrations in the soil surrounding the roots of the trees in each experimental plot. The variation in mean gas concentrations measured once a month was statistically analysed for significance. The mean atmospheric pressure and

mean daily temperature on the days of gas monitoring were compared with mean percentage gas measured. This was carried out to determine if meteorological conditions effected the gas concentrations in the root zone. The atmospheric pressure and temperature were measured by the South African Weather Bureau at Durban International Airport, approximately 20km south of the landfill.

Soil temperatures 30cm below the surface were taken using a Sharp YFE YF-1062 digital thermometer and compared with ambient air temperature. This was done by inserting the digital thermometer into each gas probe on the three plots.

Further gas measurements were made to investigate the relationship between concentration and depth. Methane, carbon dioxide and oxygen concentrations were measured at 3 soil depths at 4 different points on control plot and landfill plots. This was done by placing 3 different lengths of gas samplers within a $0.25m^2$ area at each of the 4 sampling points on the plots. Thus, gas was sampled from three depth intervals, namely 10-20cm, 25-30cm and 40-50cm. Methane, carbon dioxide and oxygen concentrations were measured using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser on 4 separate days. The relationship between the gases measured and the soil depth was analysed using regression analysis in order to determine the equation of best fit for the data.

4.2.5 Soil chemical analysis

Each plot was divided equally by area into four sub-plots, from each sub-plot three soil samples were taken at random and pooled together. The soil samples from each plot were taken at a depth of 5-10cm sealed into plastic bags and thoroughly mixed. From these samples, sub-samples were taken for soil analyses. Four sub-samples from each plot were

sent to the KwaZulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: extractable P ; K ; Ca ; Mg ; Zn ; Mn ; Extractable Acidity; Total cation; Acid saturation; pH (KCl); organic carbon percentage; and percentage clay (Hunter, 1974). This gave a basic set of soil variables for the comparison between the experimental plots.

Soils were air dried, large stones removed, and then lightly ground to breaks soil clods and sieved through a 1mm sieve. The sample density of each sample was calculated by taking a known volume of soil and determining its mass. The sample density was used to determine the mass of soil used for each test, which was carried out on a volume basis. The measured concentrations of each soil constituent were converted using the sample density from mg/ℓ to mg/kg. The pH of the soil was determined using a pH electrode placed in a $10cm^3$ soil: $25cm^3$ 1M KCl suspension which was mixed and allowed to stand for 60 minutes.

Extractable calcium, magnesium and acidity were determined from $2.5cm^3$ soil: $25cm^3$ 1M KCl solution which was stirred for 10 minutes and then filtered through Whatman No. 1 filter paper. The reagent used for Ca and Mg determination was a strontium solution consisting of 380g $SrCl_2.6H_2O$ added to 2 litres of concentrated HCl and made up to 40 litres with de- ionized water. A $5cm^3$ aliquot of the KCl soil filtrate was diluted five times to $25cm^3$ and added to $20cm^3$ of the strontium solution, this was then used to determine Ca and Mg by atomic absorption with the following instrument settings: Ca was determined at 422.7nm, current of 3.7 mA and a slit width of 0.5nm; Mg was determined at a wavelength of 589.6nm, current of 3.5mA and slit width of 0.5nm. The reagents used for extractable acidity determination from the KCl soil extract included a solution of phenolphthalein. This was made up by adding 5g phenolphthalein powder into $500cm^3$ ethanol and adding

approximately 500cm^3 water to make up a 1 litre stock solution, a diluted phenolphthalein solution was then made by adding 300cm^3 of the stock to 10ℓ of de-ionized water. A 10cm^3 aliquot of the KCl soil extract was diluted two times to 20 cm^3 and added to 10cm^3 of de-ionized water containing 2-4 drops of the diluted phenolphthalein solution. This was titrated with 0.005M NaOH to determine the centimoles of acidity per litre of soil using the following equation:

$$\frac{\text{No. cm}^3\text{ 0.005M NaOH - No. cm}^3\text{ reagent blank}}{2} = \text{centimole of acidity per litre of soil (cmol(+)/l)}$$

Extractable phosphorus, potassium, zinc and manganese was determined using an extracting solution prepared by dissolving 197.6g NH_4HCO_3 in de-ionized water, dissolving 37.2g disodium salt of ethylenediaminetetra-acetic acid (EDTA) in de-ionized water, dissolving 3.7g NH_4F in de-ionized water, and measuring out 100cm^3 of concentrated solution of Superfloc (grade N100) consisting of 10g of the flocculant in 2000cm^3 of water. The above mentioned solutions were mixed into 5ℓ of distilled water and brought to a final volume of 10ℓ. The pH of the prepared ammonium bicarbonate extracting solution was then adjusted to 8 using a strong ammonia solution.

The phosphate colour reagent was prepared by placing 2g antimony potassium tartrate in 800cm^3 distilled water and mixing with 300cm^3 of concentrated H_2SO_4 and allowed to cool overnight. 15g of ammonium molybdate was dissolved in 600cm^3 of water and added to the acid antimony potassium tartrate solution and brought to a volume of 1ℓ using distilled water. On the day of use 150cm^3 of the molybdate solution was diluted to 1ℓ with a

solution containing 1g gelatine per litre of warm water, and 1g of ascorbic acid was added and mixed. Phosphate standards were made by dissolving 0.4390g KH_2PO_4 in 975cm^3 de-ionized water, and adding 25cm^3 7N H_2SO_4. This provided a stock solution containing 100 mgℓ^{-1} P. From the P stock solution 0, 10, 20, 40 and 60cm^3 were taken and made up to 1 litre with the ammonium bicarbonate extracting solution. This provided phosphate standards of 0, 1,2,4,6 mgℓ^{-1} P.

The potassium standards were made by taking the stock and making it up to 1ℓ, thus a concentration of 600 mgℓ^{-1}. Zero, 10, 20, 50 and 100cm^3 of the K solution were made up to 1ℓ using the ammonium bicarbonate extracting solution. This provided potassium standards of 0, 6.1, 12.2, 31.6 and 66.7 mgℓ^{-1}

Zinc and manganese standards were made up by taking 50cm^3 of 1000 mgℓ^{-1} Zn and Mn atomic absorption standards and adding to 9950cm^3 distilled water, thus a concentration of 50 mgℓ^{-1}. Zero, 2,4,10, and 20cm^3 of the Zn and Mn 50 mgℓ^{-1} stock solution was made up to one litre with ammonium bicarbonate extracting solution. This provided zinc and manganese standards of 0, 0.1, 0.2, 0.5, and 1 mgℓ^{-1}.

The aforementioned set of reagents were used for determining extractable P, K, Zn and Mn with the following procedures. A 2.5cm^3 scoop of each soil sample was shaken with 25cm^3 of ammonium bicarbonate solution for 10 minutes. They were then filtered through Whatman No. 1 filter paper and kept at a constant temperature of 22°C. Extractable P was determined by taking a 2cm^3 aliquot of the filtrate, adding 8cm^3 distilled water and 10cm^3 of ammonium molybdate colour reagent. The same dilution was added to the P standards

and after 40 minutes the absorbance values at 670nm with a spectrophotometer were measured. Extractable K was determined by taking $5cm^3$ of the ammonium bicarbonate soil filtrate and adding $20cm^3$ of de-ionized water. The same dilution was added to the potassium setting standards and K was determined by atomic absorption with the following settings: wave length(λ)=766.5nm; current= 5,0mA; slit width=1.0nm. Extractable Zn and Mn were determined on the remaining undiluted ammonium bicarbonate soil filtrate with the following atomic absorption settings: Zn: wave length= 213.9nm ; Mn wave length= 279.5nm and for both Zn and Mn a Current= 5.0mA ; Slit width= 1.0nm.

The percentage organic carbon and percentage clay content of air-dried soil samples was determined by absorbance of light in the infrared region of the spectrum. Nineteen different wavelengths in the near infrared region of the spectrum were used to scan the soil samples and the absorbances were recorded on computer. The absorbances were then used in a set of formulas used to calculate organic carbon and clay percentages. The formulas were obtained by scanning a range of soils that had been analysed using standard wet chemistry methods for % carbon and % clay determination. A multiple linear regression analysis was performed to establish the relationship between the relevant soil constituent and the absorbances of the wavelengths best suited to analyse a particular constituent.

Using the University of Natal facilities sub samples of soil were also analysed using X-ray fluorescence spectrometry for total Si; Al; Fe; Mn; Mg; Ca; Na; K; Ti; P; Nb; Y; Rb; Zr; Sr; U; Th; Zn; Cu; Ni; Cr; V; La; Ba; Sc; S; Cd; Pb; Ga; Co; Ce; Nd; As. The samples were milled to less than 40 μm particle size. After mixing the residue with 5.0 g lithium metaborate and 25 mg lithium bromide, it was fused at 1200 °C for 20 min. The resultant

samples were analysed by wavelength dispersive x-ray fluorescence spectrometry using a Phillips PW1480 spectrometer.

Three, approximately 50g, samples of air-dried, sieved soil from each plot was saturated with de-ionized water and allowed to stand for 24 hours. The samples were then centrifuged using a Beckman G.P. centrifuge (No. 355953) at 3700rpm (relative centrifugal field = 2127.4) for 30 minutes to extract the supernatant. (Jackson, 1962). The conductivity of the supernatant was measured using a Crison MicroCM 2201 conductivity meter corrected to 25°C.

4.2.6 Soil physical analysis

The mean soil moisture content of each plot was calculated by loss of weight after oven drying at 105°C and expressed as a percentage. This was done using six fresh 10g sub-samples of soil from each plot. (Grimshaw, 1989). The remaining soil from these samples was air-dried. It was then lightly ground using a mortar and pestle so as to break up the clods and then sieved through a 2mm sieve. The weight of stones removed by sieving in relation to the original weight of air dried soil was expressed as the percentage stone content of the soil sample (Grimshaw, 1989).

4.2.7 Data analysis

Statistical analysis of the data was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data were analysed using an analysis of variance. If there was a significant difference ($p<0.05$) in data sets with more than two sample variables then

Scheffe multiple range test was performed by constructing intervals for pair-wise differences of means to determine which differences were significant ($p<0.05$). However, this was only done if the residuals of the data were normally distributed, as tested using a Kolmogorov-Smirnoff test for normality, ($p>0.05$). If the data were not normally distributed, and transformations were unsuccessful, a Kruskall-Wallis analysis for non-parametric data was used. If a significant difference ($p<0.05$) was found using the Kruskall- Wallis analysis then a Mann-Witney U test was used to analyse the data in a pair-wise manner so as to determine which differences were significant ($p<0.05$) (Zar, 1984). The relationship between two variables was evaluated also using a scatter plot and Pearson's Product-moment correlation analysis and regression analysis (Zar, 1984).

4.3 RESULTS

4.3.1 Soil gases

No significant change ($p<0.05$) in methane, carbon dioxide and oxygen concentrations in root zone, for all the plots, individual plots or individual gas samplers was found in relation to atmospheric pressure, daily temperature, or month. However, there was very little variation in the temperature and pressure between the different days on which gas measurements were taken. The maximum and minimum atmospheric pressure recorded for the days on which gas measurements were made were 1026.9 Mpa and 1010.9 Mpa respectively, with a mean value of 1016.2 Mpa (Std. error ± 1.19). The maximum and minimum temperatures for the days on which gas measurements were made were 25.8°C and 16.2 °C respectively, with a mean value of 21.0°C (Std. error ± 0.94). Therefore, it can be concluded that the temperature and pressure ranges experienced during the experiment did not account for the observed changes in methane, carbon dioxide or oxygen soil concentrations.

Figure 4.1 shows the carbon dioxide, methane and oxygen concentrations found in the root zone (50cm soil depth) of the control and experimental plots, calculated from the measurements taken from all of the gas samplers on each plot throughout the experimental period (14 months). Carbon dioxide was found in the root zone of all of the plots, however it was significantly ($p<0.01$) higher in the plots on the landfill. Of the two plots on the landfill, the plot without topsoil had a significantly ($p<0.01$) higher carbon dioxide concentration (48.3%) than that with topsoil (25.6%) (Figure 4.1). The presence of methane was only found in the plots situated on the landfill, with significantly ($p<0.05$) lower concentrations on the topsoil plot (22.3%) in comparison to the plot without topsoil (41.9%). The concentration of oxygen within the control plot (16%) was significantly ($p<0.05$) higher than in the landfill topsoil plot (3.2%) and the landfill plot without topsoil (0.6%). However, no significant difference ($p>0.05$) in oxygen concentrations between the two landfill plots was found (Figure 4.1).

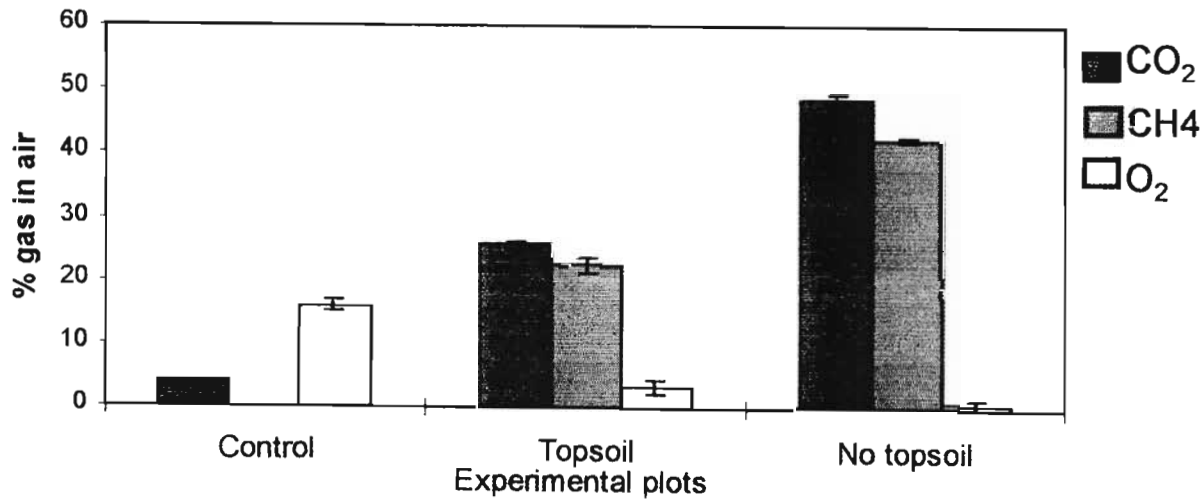


Figure 4.1: Carbon Dioxide, methane, and oxygen in the root zone of each plot measured on 14 occasions during the experiment. Results are mean values with standard errors

Carbon dioxide is found in relatively low concentrations (mean=3.9%) in the soil atmosphere of healthy aerobic soils, as shown in the control plot (Figure 4.1). The significantly higher concentrations of carbon dioxide and the presence of methane in the soil atmosphere of the experimental plots on the landfill, showed that the waste below these plots was having a significant effect on the composition and concentrations of gases in the soil atmosphere. The oxygen concentrations in the soil of the control plot were close to ambient air concentrations with low carbon dioxide and no methane. However, oxygen concentrations on the landfill plots were almost zero with high concentrations of carbon dioxide and methane. These results showed that anoxic soil conditions prevailed on the landfill and that it was related to the increased carbon dioxide and methane in the soil atmosphere.

By the comparison of the two plots on the landfill the application of topsoil was found to significantly ($p<0.01$) decrease the concentrations of carbon dioxide and methane found within the soil atmosphere (Figure 4.1). This difference in landfill gas concentrations was unlikely to be due to coincidental spatial variation in gas concentrations, as the area for the field experiment was found to be relatively homogenous in terms of landfill gas before the topsoil was applied (Table 4.1). The ratio of methane to carbon dioxide on the landfill plot with topsoil was 0.77 (Std error 0.06) and on the plot without topsoil was 0.87 (Std error 0.01). These ratios were not significantly ($p>0.05$) different, showing that although the volume of each gas in the topsoil layer was lower the relative composition of the gas had not changed. This is interesting as it suggests that the oxidation of methane into carbon dioxide was not the primary cause of lower methane in the topsoil plot (i.e. CO_2 levels, proportionally did not rise).

The relationships between CO_2, CH_4, and O_2 for all the individual gas measurements made on the control and the experimental plots are shown in Figures 4.2, 4.3 and 4.4. The landfill plot which received topsoil had a wide range of methane and carbon dioxide concentrations and this plot accounted for most of the variation in the whole data set. The data points along the y-axis of Figures 4.2 and 4.3 show that no methane was found in the control plot soil. Further regression and correlation analysis of the relationship between the gases measured in the landfill soil atmosphere was conducted by excluding the methane, carbon dioxide and oxygen data from the control plot from the statistical analyses (Table 4.2). However, to satisfy the assumption of normality of residuals for these tests, both the dependent and independent variables were transformed using an arcsine transformation where necessary. That being the proportions of each gas measured (P) expressed as the transformed value A (=arcsin $\sqrt{P}$). Conclusions from these results are made with reference to the Figures (4.2-4.4) and Table 4.2.

The carbon dioxide concentrations appeared to increase with increasing methane concentration (Figure 4.2). The methane and carbon dioxide data had a positive linear relationship ($y=0.56x + 8.8$, $R^2=0.73$, $p<0.01$) (Table 4.2). However, methane was only found in the soil atmosphere when carbon dioxide was in excess of 8.8%, as indicated by the y intercept of the regression analysis (Table 4.2).

Table 4.2: Linear Regression and Pearson's Product-Moment Correlation for the relationship between methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) in the soil atmosphere.

Relationship	Y intercept (transformed data)	y intercept [a] (Back transformed)	Slope	R^2	Correlation coefficient
[b] CH_4 (x) *versus* CO_2 (y)	0.30± 0.02	8.8%	0.56± 0.03	0.73	0.86*
[b] CH_4 (x) *versus* O_2 (y)	0.26± 0.02	6.6%	-0.27± 0.02	0.43	-0.66*
[c] CO_2 (x) up to 23% *versus* O_2 (y)		14.9%	-0.60± 0.1	0.54	-0.73*
[c] O_2 (x) *versus* CO_2 (y) up to 23%		20.7%	-0.89± 0.14	0.53	-0.73*

[a] [sin (arcsin transformed value x 180 ÷ PI)]^2 x 100
[b] These data were arcsin transformed
[c] The data were normally distributed, therefore there was no need for a arcsin transformation
* $p<0.01$

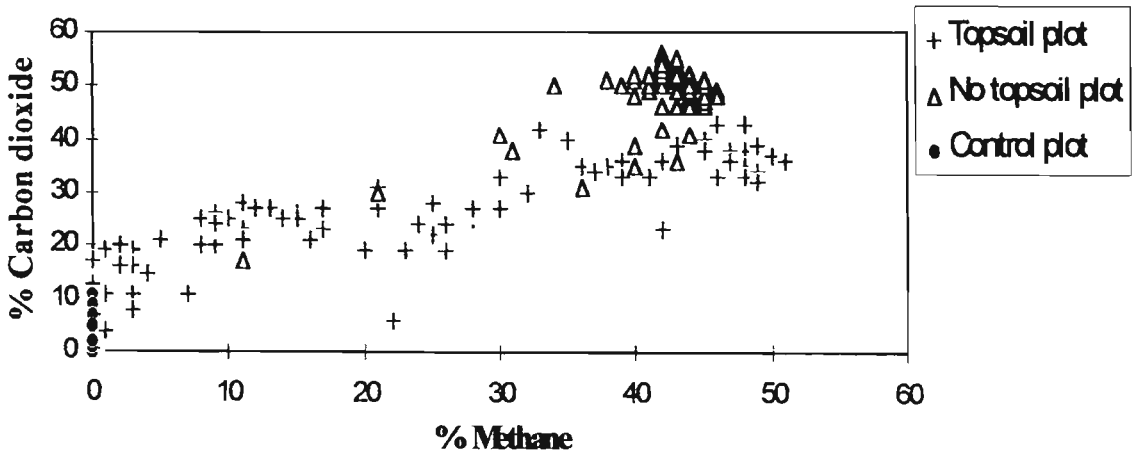


Figure 4.2: Relationship between methane and carbon dioxide in the root zone of the trees planted on the control and the experimental plots (Data points for each experimental plot given a different symbol).

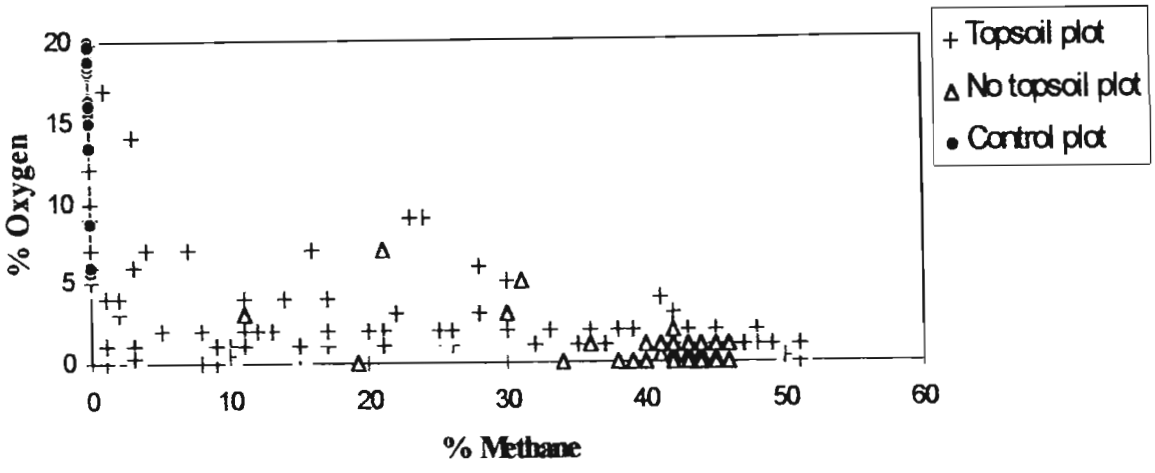


Figure 4.3: Relationship between methane and oxygen in the root zone of trees planted on the control and experimental plots (Data points for each experimental plot given a different symbol).

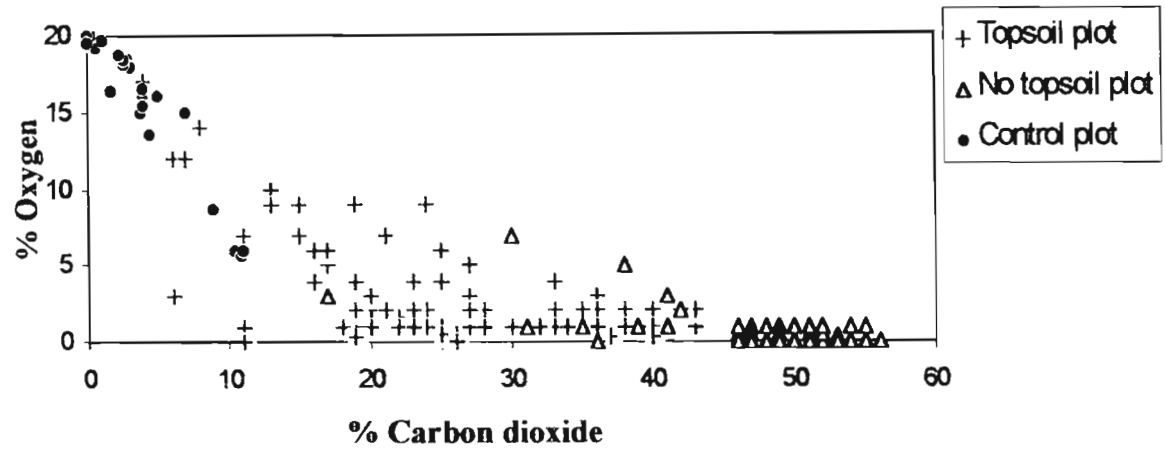


Figure 4.4: Relationship between the carbon dioxide and the oxygen in the root zone of the trees on the control and experimental plots (Data points for each experimental plot given a different symbol).

Methane and oxygen had a negative linear relationship ($y=-0.27x + 6.6$, $R^2=0.43$, $p<0.05$) (Table 4.2), showing that oxygen was lowered with increasing methane (Figure 4.3). The slope of the regression line was low (-0.27) indicating a very small change in oxygen with

increasing methane (Table 4.2). It was also noted that oxygen was already reduced to 6.6% before methane was detected, as indicated by the y intercept (Table 4.2).

The relationship between carbon dioxide and oxygen showed that oxygen concentrations were rapidly reduced, from ambient air concentrations to almost zero, as carbon dioxide increased to approximately 23% (Figure 4.4). Oxygen concentrations then remained close to zero and carbon dioxide concentrations continued to increase (Figure 4.4). A linear regression of the initial decline in oxygen, up to a carbon dioxide concentration of 23%, was calculated. A negative linear relationship (R^2=0.54) ($p<0.05$) with a very steep gradient (slope= -0.60) of decline was found (Table 4.2). This quantified the rapid depletion of oxygen, from ambient air concentrations. A further regression analysis with carbon dioxide as the dependant variable and oxygen as the independent variable showed that for these data oxygen was totally depleted at 20.7% carbon dioxide concentration (Table 4.2).

In summary, these results of individual gas measurements showed that methane was only detected in the soil when carbon dioxide concentrations were in excess of 8.8% and oxygen levels were already depleted below 6.6%. The ambient oxygen concentrations were reduced to zero when carbon dioxide had increased to 21%.

The results of the analysis of the soil gas composition at different soil depths within the control and experimental plots are shown in Figures 4.5; 4.6 and 4.7. As expected the concentration of methane and carbon dioxide increased and oxygen levels decreased with soil depth on the landfill experimental plots. A similar relationship was found on the control plot, however the gas concentrations measured were not as extreme and the lack of

underlying anaerobic waste decomposition resulted in no methane. The control plot trees had a maximum rooting depth of 70cm which coincided with an extrapolated oxygen concentration of 13% and carbon dioxide level of 3% (Figure 4.5).

The methane, carbon dioxide and oxygen concentration gradients in the landfill cover material were less steep than that found in the topsoil placed on the landfill. Thus there was higher methane and carbon dioxide and lower oxygen concentrations at shallower soil depths in the landfill cover material relative to the topsoil layer (Figures 4.6 and 4.7). This was probably due to relatively high compaction and poor soil structure of the landfill cover material, allowing for little atmospheric dilution of the landfill gas infiltration from depth. The shallower rooting depths on the landfill with or without a topsoil layer can be explained by the soil atmosphere conditions. On the landfill topsoil plot the maximum rooting depth of 40 cm coincided with a methane concentration of 53%, 20% carbon dioxide and 2% oxygen. On the landfill plot without topsoil, the maximum rooting depth of 20 cm coincided with a methane concentration of 57%, 27% carbon dioxide and 1% oxygen. Although the maximum rooting depths on the two landfill plots were different, it is interesting to note that soil gas composition at maximum rooting depth was reasonably similar. This suggests that the composition of the soil atmosphere was the key factor determining rooting depth.

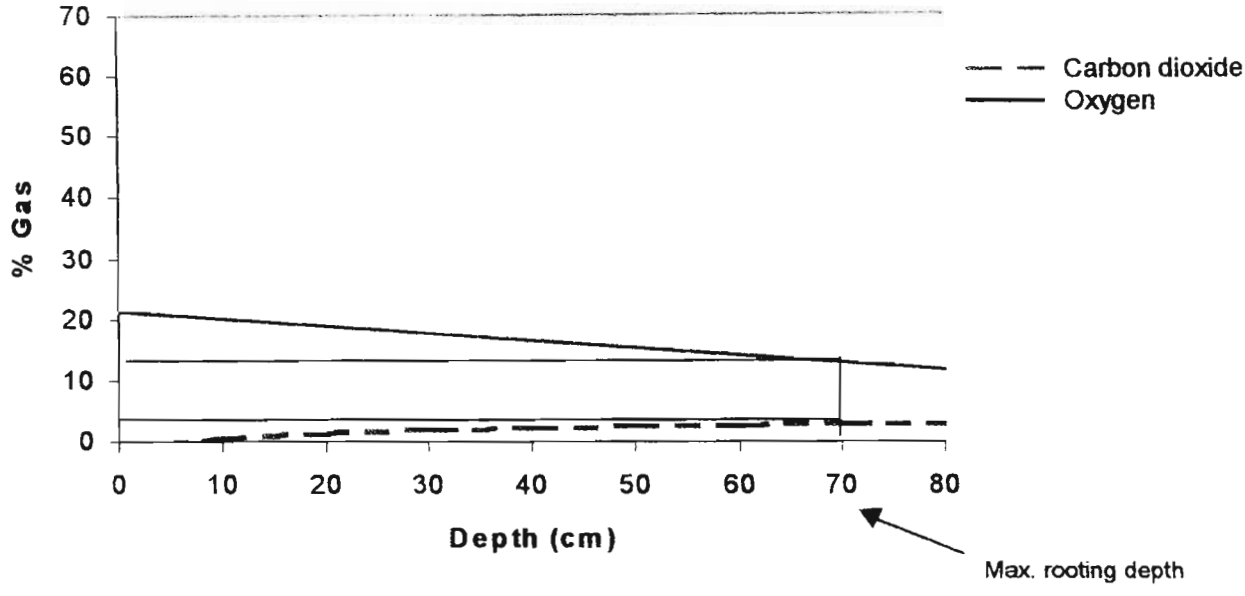


Figure 4.5: Regression models of gas concentrations measured with soil depth in the control plot topsoil layer. Carbon dioxide model equation: $y = 1.2078\ln(x) - 2.1747$, $R^2 = 0.94$, $p<0.05$. Oxygen model equation: $y = -0.1167x + 21.106$, $R^2 = 0.87$, $p<0.05$.

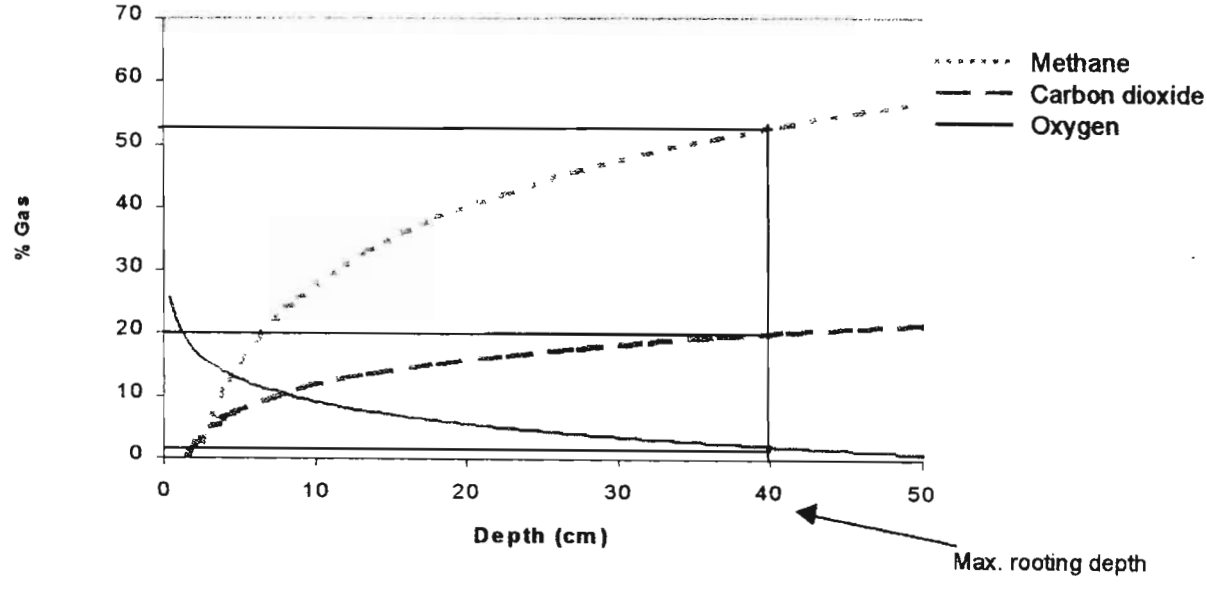


Figure 4.6: Regression models of gas concentrations measured with soil depth in the topsoil placed on the landfill (Topsoil plot). Methane model equation: $y = 17.906\ln(x) - 13.324$, $R^2=0.68$, $p<0.05$. Carbon dioxide model equation: $y = 6.0159\ln(x) - 1.9091$, $R^2=0.53$, $p<0.05$. Oxygen model equation: $y = -5.0934\ln(x) + 20.823$, $R^2 = 0.45$, $p<0.05$

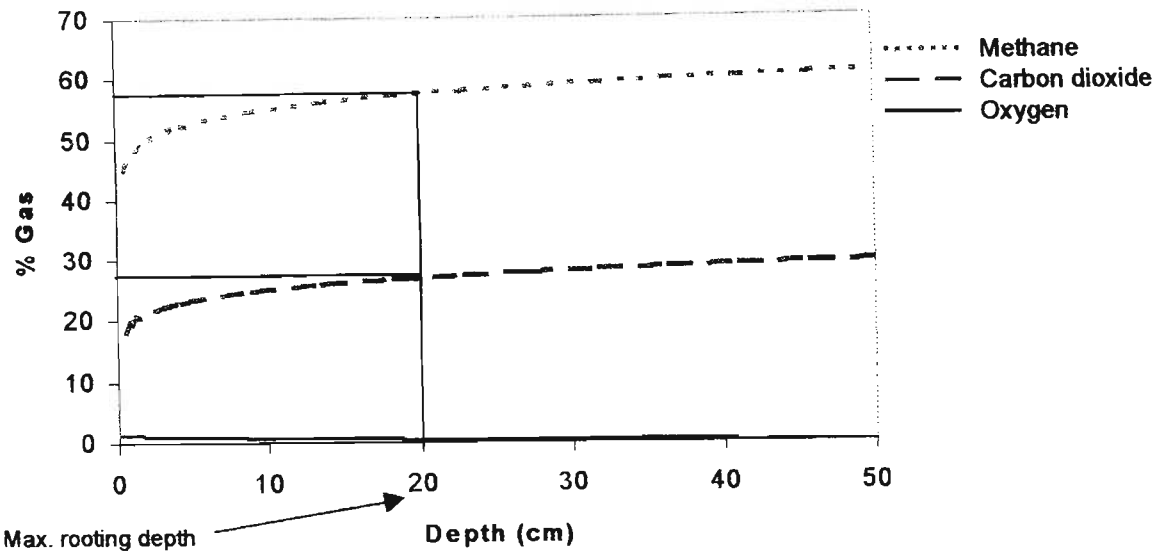


Figure 4.7: Regression models of gas concentrations measured with soil depth in the in the landfill cover material (No topsoil plot). Methane model equation: $y = 47.819\ x^{0.0617}$, $R^2 = 0.12$, $p < 0.05$. Carbon dioxide model equation: $y = 20.036\ x^{0.0999}$, $R^2 = 0.30$, $p < 0.05$. Oxygen model equation: $y = 1.2119e^{-0.0496x}$, $R^2 = 0.29$, $p < 0.05$.

4.3.2 Soil temperature

The control plot had the lowest mean temperature of 19°C which was the same as the ambient atmospheric temperature followed by the landfill plot with topsoil, which had a significantly ($p<0.01$) higher temperature of 20.7°C. The landfill plot without topsoil had the significantly ($p<0.01$) highest mean soil temperature of 22.9°C.

The relationship between temperature and landfill gas in the soil of the control and experimental plots was assessed using a regression analysis. No significant variation in the gas measurements taken at different times during the experimental period was found, therefore a mean carbon dioxide, methane and oxygen value for all the measurements taken for each of the gas samplers was calculated. The mean gas measurement for each gas

sampler on all of the plots was compared with a single soil temperature reading made at each gas sampler. The ambient atmospheric air temperature at the time of the temperature measurements was 19°C. The relationship between the gases measured and the soil temperature was analysed using a exponential, linear, logarithmic and reciprocal regression in order to determine which equation fitted the data best in terms of the respective R^2 values.

A reciprocal regression (1/y= a+bx) was found to best describe the relationship that methane and carbon dioxide had with soil temperature. Methane had a R^2=0.70 and carbon dioxide a R^2=0.75. The temperature of the soil increased with the increasing concentrations of carbon dioxide (Figure 4.8) and methane (Figure 4.9) present. In the case of the relationship between oxygen and temperature in the soil (Figure 4.10), the temperature decreased exponentially with increasing oxygen concentrations (R^2=0.67). It can be concluded from the temperature results that methane and carbon dioxide were warm gases and were responsible for raising the soil temperatures above that of the ambient air temperature. The fact that oxygen has an opposite relationship with soil temperature was probably because oxygen concentrations were found to decrease with increasing methane and carbon dioxide concentrations (Figure 4.3 and 4.4). Therefore, the higher the oxygen levels, the lower the carbon dioxide and methane levels, and thus the lower the soil temperatures and the nearer the soil temperature would be to the ambient air temperature.

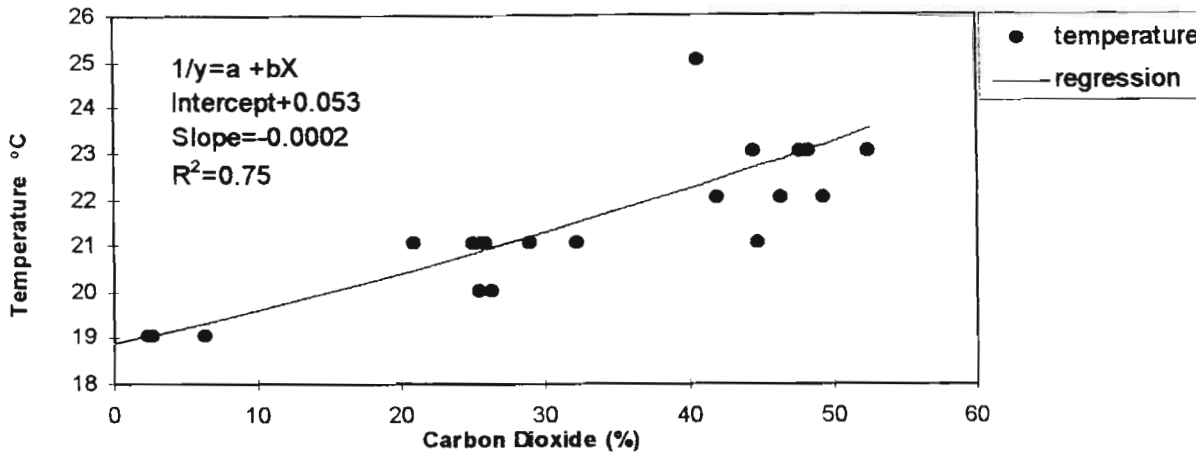


Figure 4.8: Relationship between % carbon dioxide and root zone temperature of the experimental plots

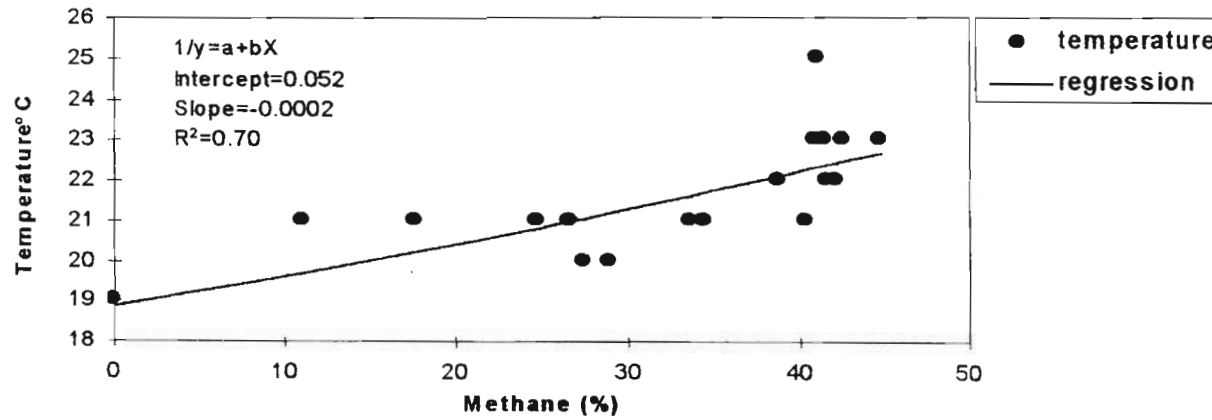


Figure 4.9: Relationship between % methane and root zone temperature of the experimental plots.

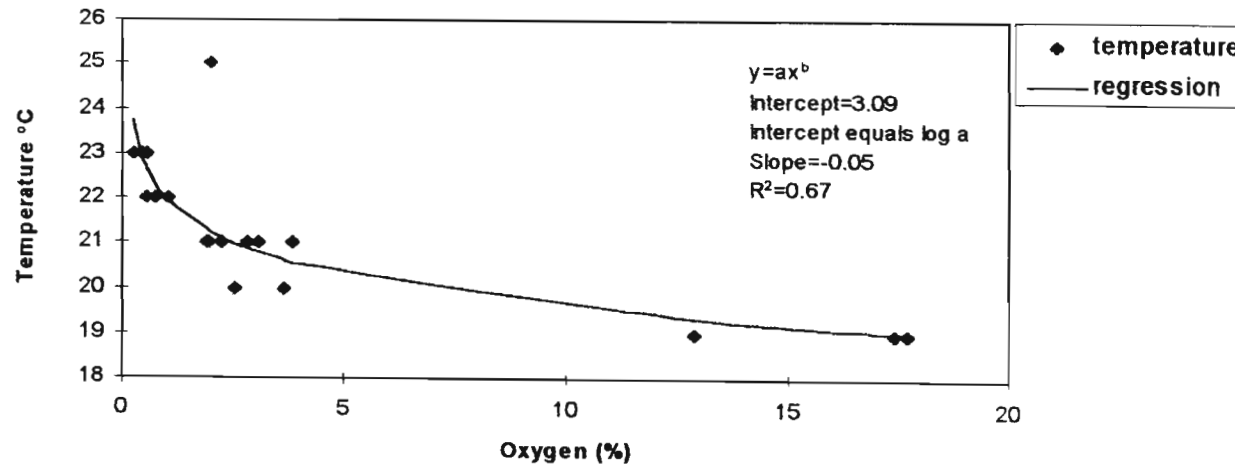


Figure 4.10: Relationship between % oxygen and root zone temperature of the experimental plots.

4.3.3 Soil chemical and physical characteristics

The measurement of the extractable (ammonium bicarbonate, pH 8) nutrients P, K, and Ca indicated that the landfill cover material (No topsoil plot) was not deficient in these nutrients by comparison to the topsoil used on the control and the experimental plot (Table 4.3). No significant differences ($p>0.05$) were found between the plots for P and Ca. However, K concentrations were significantly ($p<0.01$) (six fold) higher in the landfill cover material by comparison to the topsoil on the landfill topsoil plot and the control plot (Table 4.3). Mg concentrations were significantly ($p<0.05$) lower in the landfill cover material by comparison to the two plots with topsoil. There was no significant difference ($p>0.05$) in percentage clay and percentage organic carbon between the control and the experimental plots. There was no significant ($p>0.05$) difference in extractable acidity in any of the plots, however, the pH and conductivity of the plot on the landfill without topsoil was significantly ($p<0.01$) higher than the landfill plot with topsoil and the control plot.

No significant differences in the 13 soil variables in Table 4.3 were found between the topsoil on the control plot, and the topsoil on the landfill, except for soil moisture and manganese concentrations. The control plot, landfill plot with topsoil and the landfill plot without topsoil all had significantly ($p<0.01$) different soil moisture levels (Table 4.3). The control plot had the highest soil moisture by comparison to the plots on the landfill. The topsoil on the landfill had 1.5% less moisture than the control plot and a further 1.7% less moisture on the plot without topsoil was found.

Table 4.3: Physical and chemical properties of soil samples collected from the three experimental plots[1].

Parameter	Control plot	Topsoil Plot	No topsoil plot
Extractable Mn (mg/kg)	4.76 ±0.32[1] a[2]	31.02 ±3.1 b	22.49 ±2.6 b
Extractable Zn (mg/kg)	5.8 ±0.16 a	10.60 ±1.66 a	16.78 ±4.3 a
% moisture (by weight)	11.80 ±0.43 a	10.32 ±0.26 b	8.70 ±0.23 c
% Stone (by weight)	17.81 ±1.6 a	18.77 ±2.3 a	51.59 ±1.6 b
Extractable P (mg/kg)	16.4 ±0.7 a	18.1 ±0.7 a	12.7 ±2.7 a
Extractable K (mg/kg)	32.4 ±0.6 a	36.6 ±2.7 a	168.7 ±25.1 b
Extractable Ca (mg/kg)	1119.6 ±38.2 a	1003.8 ±76.8 a	990.67 ±86.5 a
Extractable Mg (mg/kg)	246.0 ±18.9 a	237.7 ±13.1 a	168.3 ±15.0 b
Organic carbon %	1.93 ±0.07 a	2.37 ±0.12 a	2.17 ±0.49 a
Clay %	24 ±0.58 a	23.33 ±1.20 a	26.33 ±3.28 a
Extrac. Acidity (Cmol/kg)	0.086 ±0.006 a	0.085 ±0.007 a	0.097 ±0.009 a
pH	7.43 ±0.11 a	7.24 ±0.05 a	8.14 ±0.12 b
Conductivity (mS/cm)	0.85 ±0.08 a	1.12 ±0.64 a	3.74 ±0.098 b

[1]Standard error of the mean (n=4)

[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Sheffe multiple range test ($p<0.01$), except for magnesium which was $p<0.05$.

An interesting difference in the chemistry of the topsoil on the landfill was a six fold higher ($p<0.01$) extractable (ammonium bicarbonate, pH 8) Mn concentration by comparison to the control plot (Table 4.3). One explanation for this is that there was an increase in Mn concentration in the topsoil after placement on the landfill. Interestingly, there was no significant difference ($p<0.01$) in Mn between the plots with and without topsoil on the landfill. Zinc concentrations were also found to have almost doubled in the topsoil placed on the landfill by comparison to the control, however the differences were not statistically significant ($p= 0.07$). Considering that the topsoil used on the control plot and that used on the landfill plot came from the same stock pile it can be concluded that the significant increase in Mn and possibly the increased amount of Zn was probably not due

to sampling. This indicates that the changes in the topsoil on the landfill may be due to an interaction between the topsoil and the underlying waste material below the plot.

A possible source of the high Mn levels may be leachate contamination of the soil. However, in this investigation drainage lines were installed to prevent leachate causing surface contamination of the experimental plots and there was no visual evidence of leachate contamination. The upward migration, by capillary action, of moisture, carrying Mn in solution, was also unlikely due to the high compaction and poor soil structure of the underlying waste and cover material. It may be possible that the upward migration of warm landfill gas carried a Mn condensate which was deposited in the topsoil layer as the gas cooled towards the soil surface. To investigate this further soil samples on the control and landfill plots were analysed for total metal concentrations using x-ray fluorescence. The results in Table 4.4 show no significant difference in total Mn concentrations between the plots. It is also interesting to note that in terms of the other metals measured there were also no significant differences between the topsoil on the control and the topsoil on the landfill. The only significant differences were between the landfill cover material and the topsoil which was used on both the control and landfill plot. The landfill cover material was found to have significantly ($p<0.05$) lower levels of metals (Al, Na, K, P, Nb, Y, Rb, Zr,Sr, Cr, Ba, Ga) in comparison to the topsoil, thus disproving the idea that the soils on the landfill were being influenced by metal-contaminated leachate or gas condensate.

Table 4.4: Total metal concentrations measured in the soil on the control and landfill plot with and without topsoil using x-ray fluorescence spectrometry (mg Kg^{-1}).

Element	Control plot		Topsoil plot		No topsoil	
Si	335286.4	±5913.9[1]	342522	±1157.6	340157.5	±8215.1
Al	56900.2	±1605.0 a[2]	55567.1	±281.0 ab	47137.9	±3681.3 b
Fe	32824.6	±4357.2	26214.5	±579.6	25825.8	±1213.8
Mn	633.0	±42.5	581.2	±42.0	517.0	±29.0
Mg	3911.1	±109.0	3617.9	±141.8	3816.7	±313.6
Ca	8721.4	±214.8	9240.6	±413.7	12855.5	±2581.9
Na	11299.0	±173.2 a	11819.5	±237.4 a	6880.8	±1492.5 b
K	20241.2	±596.4 a	21138.3	±610.0 a	14746.8	±1726.8 b
Ti	4365.2	±85.1	4341.6	±69.1	4224.8	±118.6
P	464.0	±11.0 a	435.4	±19.8 ab	380.5	±8.9 b
Nb	14.0	±0.4 a	13.6	±0.4 ab	11.6	±0.7 b
Y	37.7	±0.7 a	37.1	±0.7 a	28.9	±2.8 b
Rb	91.8	±1.1 a	92.4	±2.1 a	69.5	±7.3 b
Zr	568.0	±11.8 a	565.4	±22.6 a	385.2	±71.1 b
Sr	145.2	±1.7 a	145.3	±0.6 a	105.6	±10.1 b
U	3.1	±0.5	2.9	±0.3	2.7	±0.4
Th	10.8	±0.7	11.9	±0.6	10.9	±0.8
Zn	82.9	±1.0	79.4	±3.4	99.3	±7.6
Cu	13.7	±1.4	11.7	±2.2	19.4	±3.1
Ni	18.2	±0.3	17.1	±0.4	19.5	±1.3
Cr	80.6	±4.8 ab	71.1	±2.2 a	95.1	±6.6 b
V	88.1	±6.1	78.0	±1.9	94.0	±5.8
La	40.2	±1.0	41.7	±2.5	38.9	±8.8
Ba	743.9	±17.1 ab	776.2	±18.5 a	638.0	±48.9 b
Sc	19.0	±1.3	17.7	±0.8	19.6	±1.2
S	560.5	±85.2	1717.8	±536.9	2104.3	±806.0
Cd	3.7	±2.2	2.4	±1.9	1.7	±1.7
Pb	46.5	±2.0	39.8	±5.4	44.9	±3.2
Ga	13.9	±0.4 a	13.7	±0.2 a	11.5	±0.8 b
Co	16.0	±1.1	14.9	±1.5	13.1	±1.3
Ce	96.8	±3.8	111.1	±7.1	86.7	±13.2
Nd	40.7	±2.7	43.5	±2.5	39.6	±4.8
As	16.1	±1.4	14.8	±1.7	21.5	±2.9

[1]Standard error of the mean (n=4)

[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Sheffe multiple range test ($p<0.05$)

4.3.4 Relative performance of tree species

The trees on the plots that were removed from the data set because of a verified cause, such as those killed by insect infestation, stolen, or broken by the wind are shown in Table 4.5.

Table 4.5: The number of trees that were removed from the data set because of a verified cause.

Cause of death	Species	Plot	No. of trees
Insect damage	*Rhus lancea*	Topsoil	2
	Combretum erythrophyllum	Topsoil	1
	Syzygium cordatum	Topsoil	1
	Erythrina lysistemon	No topsoil	1
Wind damage	*Syzygium cordatum*	Topsoil	1
	Rhus lancea	Control	1
	Acacia xanthophloea	No topsoil	1
Stolen	*Barringtonia racemosa*	Topsoil	4

Table 4.5 indicates that insect damage was the main cause of verifiable tree death. The predominance of insect damage on the topsoil plot was most likely the result of a random single plant infestation that spread, and is unlikely to be related to differences in environmental conditions between the plots. Wind damage on the landfill plots could be expected, as on the landfill there was very little vegetation or topographical features to break the flow of air. The stealing of four *Barringtonia racemosa* from the fenced topsoil plot can only be used to illustrate the diversity of problems that can be encounted when trying to revegetate a landfill environment. Survival and health category measurements were collected for all seven *Barringtonia racemosa*, however, the trees were stolen before growth measurements were completed and, therefore, growth data for only three trees of this species from the landfill plot with topsoil were available. Theft is a general problem for landfill revegetation in South Africa. Most landfills support a population of people who

make a living from salvaging goods from the site, thus the temptation of newly planted trees which can be sold or used for medicinal purposes can be irresistible.

Tree mortality and health

Twelve trees were removed from the experimental data set for the assessment of the trees relative performance (Table 4.5). No deaths on the control plot were recorded within the first 7 months, however over the following 8 months *Acacia xanthophloea*, *Erythrina lysistemon*, *Rhus lancea* and *Acacia sieberiana* had a number of mortalities (Table 4.6). The effects of the stress of transplanting may have required a full growing season to become evident, possibly explaining the increase in mortality in the final 8 months of the experiment. *Acacia sieberiana* showed a similar increase in mortality with time on the control and experimental plots reinforcing the suggestion that transplanting stress may have contributed towards mortality. However, *Acacia xanthophloea* had a mortality that was higher on the control plot by comparison to that on the landfill experimental plots. It was also noted that the increase in mortality on the control plot, over the final eight months of the experiment for *Acacia xanthophloea*, *Erythrina lysistemon*, and *Rhus lancea* did not occur on the landfill experimental plots. This could suggest, especially for *Acacia xanthophloea*, that something other than transplanting stress might have been affecting these species on the control plot. This will be discussed in greater detail in the section on tree growth.

Table 4.6: Percentage of dead trees of each species on the control plot throughout the experimental period.

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0	0	0	0	14.3
Acacia xanthophloea	0	0	0	0	57
Barringtonia racemosa	0	0	0	0	0
Combretum erythrophyllum	0	0	0	0	0
Erythrina lysistemon	0	0	0	0	28.6
Harpephyllum caffrum	0	0	0	0	0
Hibiscus tiliaceus	0	0	0	0	0
Rhus lancea	0	0	0	0	14.3
Strelitzia nicolai	0	0	0	0	0
Syzygium cordatum	0	0	0	0	0
Mean	0	0	0	0	11.4

In terms of overall tree species mortality after 14 months the control plot experienced the lowest mortality of 11%, the landfill topsoil plot had a mortality of 23% and the landfill plot without topsoil had a mortality of 36% (Tables 4.6, 4.7 and 4.8). The higher mortality on the topsoil plot by comparison to the control plot, which received the same topsoil, suggested that changes in the soil characteristics of the topsoil on the landfill, or some other environmental variables, had a negative effect on the tree survival. However, the application of topsoil did have a beneficial effect on tree mortality, reducing it by 50% after 7 months when compared to the trees planted directly into the landfill cover material (Tables 4.7 and 4.8). It is important to note that over the final eight months, although the topsoil layer still resulted in lower tree mortality, mortality increased by 9% on the topsoil plot (Table 4.7), whilst it only increased by 4% on the no topsoil plot (Table 4.8). This may suggest that the ameliorative properties of the topsoil were reduced with time.

Table 4.7: Percentage of dead trees of each species on the Topsoil plot throughout the experimental period.

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0.0	0.0	0.0	0.0	28.6
Acacia xanthophloea	0.0	0.0	0.0	0.0	0.0
Barringtonia racemosa	0.0	0.0	0.0	0.0	0.0
Combretum erythrophyllum	0.0	0.0	16.7	16.7	16.7
Erythrina lysistemon	0.0	0.0	0.0	14.3	14.3
Harpephyllum caffrum	0.0	0.0	0.0	57.1	71.4
Hibiscus tiliaceus	0.0	0.0	0.0	0.0	0.0
Rhus lancea	0.0	0.0	0.0	0.0	0.0
Strelitzia nicolai	0.0	0.0	14.3	28.6	57.1
Syzygium cordatum	0.0	0.0	0.0	33.3	50.0
Mean			3.1	15	23.8

In terms of individual species survival on the landfill experimental plots *Hibiscus tiliaceus* and *Barringtonia racemosa* had no deaths on any of the plots (Table 4.7 and 4.8). *Combretum erythrophyllum* had only 14–17% mortality on the landfill plots. These mortality results suggested that *Barringtonia racemosa, Combretum erythrophyllum* and *Hibiscus tiliaceus* were relatively tolerant to the landfill conditions and the application of topsoil did not reduce the mortality of these species. This was confirmed by the health category data, which showed little change between the plots for these species (Figure 4.11).

Table 4.8: Percentage of dead trees of each species on the No topsoil plot throughout the experimental period

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0.0	0.0	42.9	42.9	57.1
Acacia xanthophloea	0.0	0.0	14.3	28.6	28.6
Barringtonia racemosa	0.0	0.0	0.0	0.0	0.0
Combretum erythrophyllum	0.0	0.0	0.0	14.3	14.3
Erythrina lysistemon	0.0	0.0	0.0	33.3	33.3
Harpephyllum caffrum	0.0	0.0	14.3	71.4	57.1
Hibiscus tiliaceus	0.0	0.0	0.0	0.0	0.0
Rhus lancea	0.0	0.0	14.3	28.6	28.6
Strelitzia nicolai	0.0	0.0	0.0	14.3	57.1
Syzygium cordatum	0.0	0.0	0.0	85.7	85.7
Mean	0	0	0	31.9	36.2

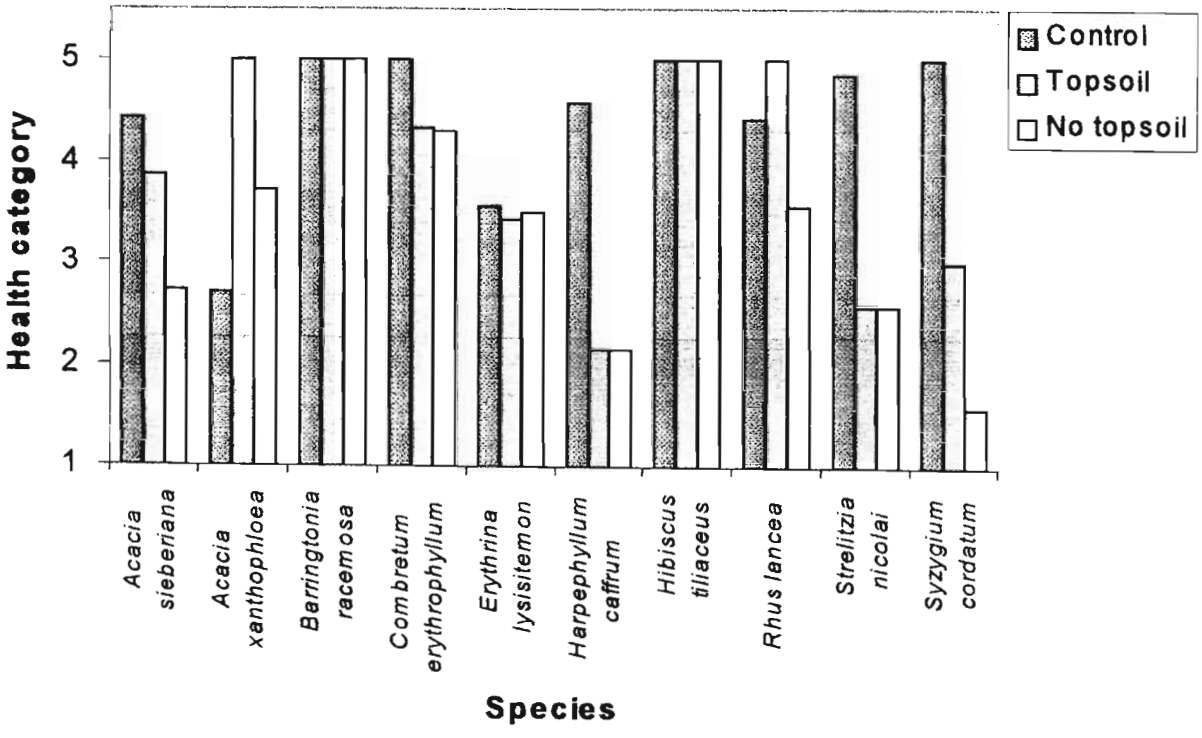


Figure 4.11: Mean health category based on the appearance of the individual plants recorded on the 6/4/98 at the end of the experiment.

Rhus lancea, Acacia xanthophloea, Erythrina lysistemon, Acacia sieberiana and *Syzygium cordatum* all appeared to benefit from the application of topsoil on the landfill and had fewer mortalities and better health on the topsoil plot in comparison with the no topsoil plot (Table 4.7 and 4.8; Figure 4.11). Of these species *Rhus lancea* and *Acacia xanthophloea* had a relatively low mortality on the no topsoil plot (28.6%) and with the application of topsoil no mortality was recorded for the 14 month experimental period. *Acacia sieberiana, Erythrina lysistemon* and *Syzygium cordatum* had a relatively high mortality on the no topsoil plot (57%), however, the application of a topsoil layer resulted in fewer mortalities 28%, 19% and 7% respectively. For some species, such as *Harpephyllum caffrum* and *Strelitzia nicolai,* which also had a high mortality on the no topsoil plot (57%), the application of topsoil did not result in fewer mortalities, in fact *Harpephyllyum caffrum* had a higher mortality on the topsoil plot (71%). It is also interesting to note that the percentage mortality of *Harpephyllum caffrum* declined by 14% between 31/7/97 and the 6/4/98, suggesting that one of the trees experienced re-growth and was not actually dead. In summary the results show that, in terms of tree mortality, there is a wide range of survival values on the landfill plots and the benefit of a topsoil layer over the landfill cover material is apparent for some species but not others.

Tree growth

The stem diameter and height growth of the individual trees was calculated by subtracting the measurements taken at the beginning of the experiment from that measured after 7 and 14 months of growth. For the whole data set the relationship between the increase in height over the experimental period and the original height when planted was investigated using linear regression. Data for all the individual trees on all the plots was used in the regression

and each species was analysed separately. The relative increase in stem diameter was assessed similarly. Surprisingly, no significant ($p>0.05$) relationship between the increase in stem dimensions and the original stem dimensions of the tree when planted was found for any of the species except *Acacia xanthophloea* and *Harpephyllum caffrum*. One may expect the increase in stem diameter and height to be greater for trees that were originally larger, because larger trees usually have greater productivity. This was true for the increase in stem diameter of *Harpephyllum caffrum*, which had a significant ($p<0.05$) positive relationship with the original stem diameter measured ($R^2=0.28$). However, the increase in stem height and diameter for *Acacia xanthophloea* had a significant ($p<0.05$) negative relationship with the original stem height and stem diameter measured, $R^2=0.23$ and $R^2=0.32$ respectively.

Even though only two species (*Harpephyllum caffrum* and *Acacia xanthophloea*) showed a relationship between the original size of the tree and size increase, the increase in stem diameter and height was expressed as a proportion of the original stem diameter and height (i.e. relative growth). It was also considered sensible to express aboveground biomass as a proportion of original stem height (i.e. relative biomass) in the following analysis.

The stem diameter and height growth as well as aboveground biomass and leaf area data was presented in two different ways for analysis. The data was firstly presented with all the dead plants included as zero values. For the stem diameter and height growth data some individual plants experienced negative growth which can be expected if plant health deteriorated and the plant tissue had lost water, these were also represented by zero values. Although the inclusion of the dead plants as zero growth could provide a good indication of individual species overall performance, it would mask information about the growth of

the surviving individuals. Therefore, the data was further presented for analysis with the dead plants removed from the data, and for the stem height and diameter data the negative growth values were also included, in order to assess the growth of the surviving trees.

With all the data for the different species combined there was no difference between the results using the two different methods of presenting the data, except for total leaf area, which will be discussed last. After the first 7 months there was a significantly ($p<0.01$) smaller height and diameter growth between the landfill plots and the control plot. The application of topsoil over the landfill cover material appeared to result in no significant ($p>0.05$) improvement on overall tree growth on the landfill (Figure 4.12). After 14 months the ameliorative effects of the topsoil started to become more apparent. There was no significant ($p>0.05$) difference in height growth between the control plot and the landfill plot which received topsoil, whilst the landfill plot which received no topsoil had a significantly ($p<0.01$) smaller growth in height (Figure 4.12a). In terms of stem diameter growth the ameliorative effects of the topsoil were also apparent after 14 months. There was a significantly better growth on the control plot then the landfill plot with topsoil however, the diameter growth of the trees planted without topsoil was significantly ($p<0.01$) less than the plots that received topsoil (Figure 4.12b).

The aboveground biomass data showed significantly reduced aboveground plant mass on the landfill when no topsoil layer was provided (Figure 4.12c). The total leaf area data also showed that plant growth was reduced by the landfill conditions, however, topsoil did not appear to reduce this effect, as seen in the stem growth and biomass results. When the dead plants were removed from the data set the total leaf area results were similar to the stem diameter and biomass results, showing reduced total leaf area on the no topsoil plot but no

significant ($p<0.01$) difference between the control and landfill topsoil plot. Therefore, the results generally suggest that tree growth was limited by the landfill conditions, however, the addition of a 1m-topsoil layer over the landfill cover material resulted in a marked improvement in growth.

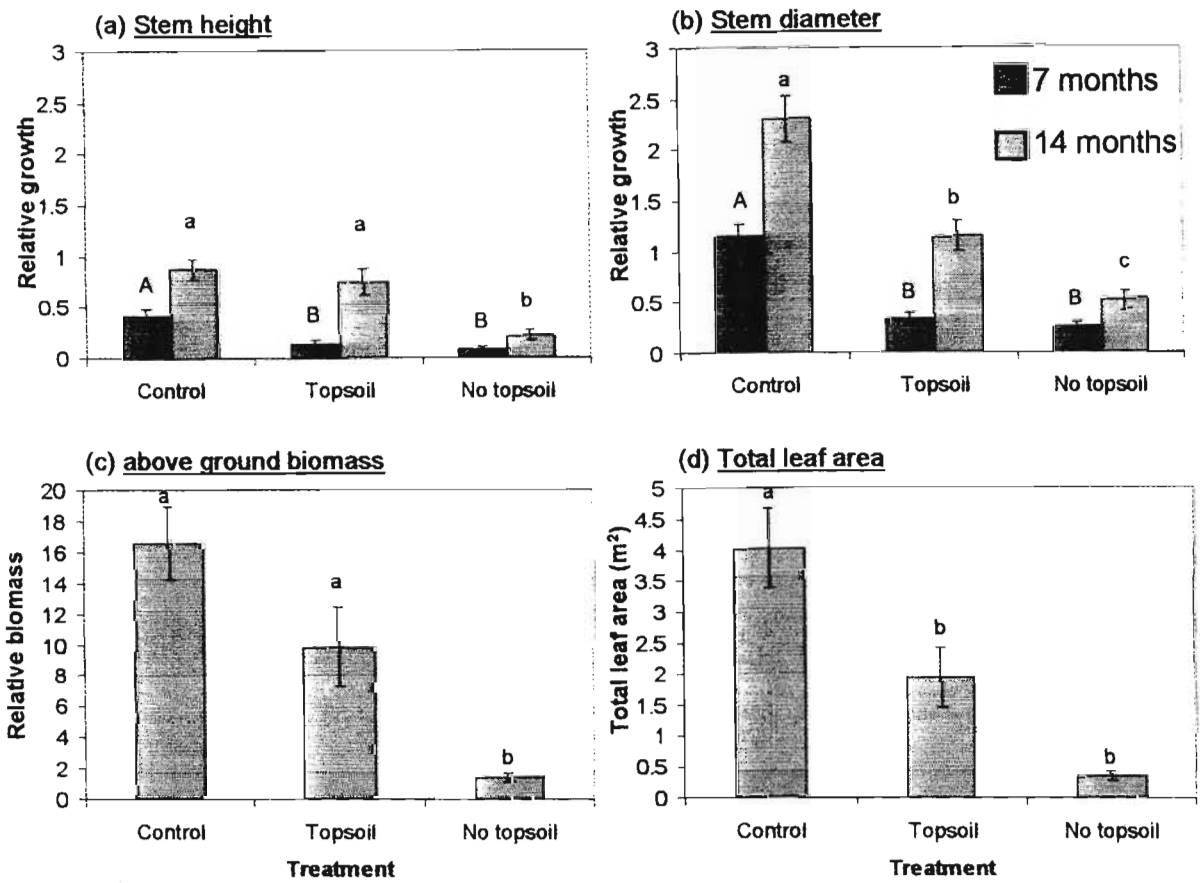


Figure 4.12: Growth response of trees to the landfill conditions. Mean relative height (graph a) and diameter (graph b) growth on control and experimental plots after 7 and 14 months. Significant differences ($p<0.01$) between plots within the 7 or 14 month category shown by a change in letter (upper case-7month; lower case-14month comparison). Mean above ground biomass after 14 months (graph c) and total leaf area after 14 months (graph d) significant differences ($p<0.01$) between treatments shown by change in letter. Error bars show standard error of the mean.

Similarly to the analysis of the combined species growth data the difference in individual species growth response data (i.e. stem diameter and height growth, biomass and total leaf area) between the two different methods of presenting the data was minimal, therefore unless specifically mentioned, the conclusions made from the two methods were the same. The key focus of the individual species stem growth results was on the data collected at 14 months, as it was considered more representative of the performance of the trees in the long term.

In the description of the results for the individual species, the species will be considered in two groups. The first group of 4 species is where there were few apparent effects of the landfill conditions (*Barringtonia racemosa; Acacia sieberiana; Erythrina lysistemon* and *Acacia xanthophloea*). The second group consists of the other six species (*Harpephyllum caffrum; Strelitzia nicolai; Syzygium cordatum; Combretum erythrophyllum; Hibiscus tiliaceus* and *Rhus lancea*). In the second group *Syzygium cordatum, Harpephyllum caffrum, and Strelitzia nicolai* showed no marked improvement in growth with the addition of topsoil on the landfill. However, *Combretum erythrophyllum, Hibiscus tiliaceus* and *Rhus lancea* all showed a marked improvement in growth when planted with the additional topsoil layer.

After 14 months the stem height growth, diameter growth, and biomass for *Barringtonia racemosa, Acacia sieberiana, Erythrina lysistemon,* and *Acacia xanthophloea* did not differ significantly ($p<0.05$) between the control and the landfill plots (Tables 4.9, 4.10, 4.11). However, the inherent variability of these measurements for *Acacia sieberiana* and *Erythrina lysistemon,* as indicated by the standard error of the mean height, diameter, biomass and leaf area data on the control were some of the highest of the 10 species

(Figure 4.13). The standard error expressed as a percentage of the mean variable measured in the control for both species, across all variables measured, was in excess of 36% (mean 50%), whilst most other species were well below 30% (Figure 4.13). Therefore the lack of significant difference in stem growth between the control plot and the landfill plots was probably due to high data variability and not necessarily indicative of a minimal growth effect of the landfill conditions. A higher number of replicates for *Erythrina lysistemon* and *Acacia sieberiana* may have resulted in better quality data for these species.

Table 4.9: Comparison of relative height increase between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative values=0 and dead plants=0.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	1.68 ±0.31[1] (7)[2] a[3]	1.76 ±0.68 (4) a	0.596 ±0.22 (7) a
Combretum erythrophyllum	0.56 ±0.06 (7) a	0.30 ±0.14 (6) ab	0.06 ±0.05 (7) b
Erythrina lysistemon	0.34 ±0.17 (7) a	0.027 ±0.02 (7) a	0.00 ±0.00 (6) a
Harpephyllum caffrum	1.29 ±0.23 (7) a	0.34 ±0.25 (7) b	0.00 ±0.00 (7) b
Hibiscus tiliaceus	1.47 ±0.16 (7) a	1.46 ±0.40 (7) a	0.37 ±0.12 (7) b
Rhus lancea	0.40 ±0.06 (6) a	0.23 ±0.07 (5) a	0.02 ±0.02 (7) b
Acacia sieberiana	0.80 ±0.37 (7) a	1.30 ±0.48 (7) a	0.50 ±0.24 (7) a
Strelitzia nicolai	0.70 ±0.14 (7) a	0.14 ±0.09 (7) b	0.04 ±0.04 (7) b
Syzygium cordatum	0.02 ±0.01 (7) a	0.00 ±0.00 (5) a	0.01 ±0.01 (7) a
Acacia xanthophloea	1.28 ±0.62 (7) a	1.86 ±0.32 (7) a	0.60 ±0.34 (6) a

[1]Standard error of the mean
[2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.10: Comparison of relative stem diameter increase between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative values=0 and dead plants=0.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	2.72 ±0.43[1] (7)[2] a[3]	2.73 ±0.90 (4) a	1.71 ±0.33 (7) a
Combretum erythrophyllum	1.91 ±0.14 (7) a	1.17 ±0.50 (6) ab	0.26 ±0.22 (7) b
Erythrina lysistemon	1.31 ±0.48 (7) a	0.44 ±0.17 (7) a	0.13 ±0.08 (6) a
Harpephyllum caffrum	0.94 ±0.16 (7) a	0.26 ±0.17 (7) b	0.01 ±0.01 (7) b
Hibiscus tiliaceus	3.39 ±0.14 (7) a	2.16 ±0.49 (7) b	0.69 ±0.18 (7) c
Rhus lancea	3.41 ±0.38 (6) a	1.62 ±0.40 (5) b	0.36 ±0.19 (7) c
Acacia sieberiana	1.02 ±0.51 (6) a	0.38 ±0.16 (7) a	0.38 ±0.31 (7) a
Strelitzia nicolai	2.87 ±0.28 (7) a	0.38 ±0.25 (7) b	0.39 ±0.24 (7) b
Syzygium cordatum	1.44 ±0.09 (7) a	0.33 ±0.21 (5) b	0.03 ±0.03 (7) b
Acacia xanthophloea	2.53 ±0.35 (7) a	3.88 ±1.84 (7) a	1.21 ±0.60 (6) a

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.11: Comparison of relative above ground biomass between the control and experimental plots i.e (biomass/ original height) after 14 months. Data presented for analysis with dead plants included as zero mass.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	4.20 ±1.3[1] (6)[2] a[3]	5.41 ±2.9 (4) a	1.01 ±0.4 (7) a
Combretum erythrophyllum	9.57 ±1.8 (7) a	5.98 ±3.1 (6) ab	1.59 ±0.8 (6) b
Erythrina lysistemon	6.10 ±3.1 (7) a	1.17 ±0.6 (7) a	0.39 ±0.2 (6) a
Harpephyllum caffrum	10.59 ±2.5 (7) a	2.17 ±1.6 (7) b	0.16 ±0.1 (7) b
Hibiscus tiliaceus	54.96 ±7.0 (7) a	47.70 ±17.0 (7) a	3.63 ±1.4 (7) b
Rhus lancea	29.82 ±4.7 (5) a	8.08 ±1.8 (5) b	1.45 ±0.8 (7) b
Acacia sieberiana	16.13 ±8.8 (5) a	9.28 ±3.3 (7) a	2.53 ±1.6 (7) a
Strelitzia nicolai	6.49 ±1.4 (7) a	1.07 ±0.7 (7) b	0.57 ±0.3 (7) b
Syzygium cordatum	10.94 ±1.6 (7) a	1.65 ±1.0 (5) b	0.27 ±0.3 (7) b
Acacia xanthophloea	18.73 ±9.4 (7) a	10.60 ±2.2 (7) a	2.18 ±1.2 (6) a

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.12: Mean total leaf area of the surviving trees of different species between the experimental plots (Dead plants removed). (Area in m^2)

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	0.60 ±0.17[1] (6)[2] a[3]	0.73 ±0.36 (4) a	0.17 ±0.08 (7) a
Combretum erythrophyllum	2.89 ±0.71 (7) a	2.65 ±1.15 (5) a	0.67 ±0.37 (6) a
Erythrina lysistemon	2.52 ±1.49 (5) a	0.42 ±0.27 (6) a	0.066 ±0.01 (4) a
Harpephyllum caffrum	2.00 ±0.46 (6) a	1.33 ±0.82 (2) a	0.04 ±0.01 (3) a
Hibiscus tiliaceus	13.82 ±1.9 (7) a	10.63 ±3.2 (7) a	1.09 ±0.53 (7) b
Rhus lancea	13.37 ±1.9 (6) a	4.07 ±0.90 (5) b	1.02 ±0.55 (5) b
Acacia sieberiana	1.18 ±0.67 (5) a	1.23 ±0.28 (5) a	0.72 ±0.38 (3) a
Strelitzia nicolai	1.81 ±0.34 (7) a	0.92 ±0.51 (3) a	0.48 ±0.16 (3) a
Syzygium cordatum	3.91 ±0.68 (7) a	1.09 ±0.52 (3) a	0.39 ±0.0 (1) a
Acacia xanthophloea	3.32 ±0.25 (3) a	0.98 ±0.23 (7) b	0.28 ±0.15 (4) b

[1]Standard error of the mean. [2]Sample size (n)

[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

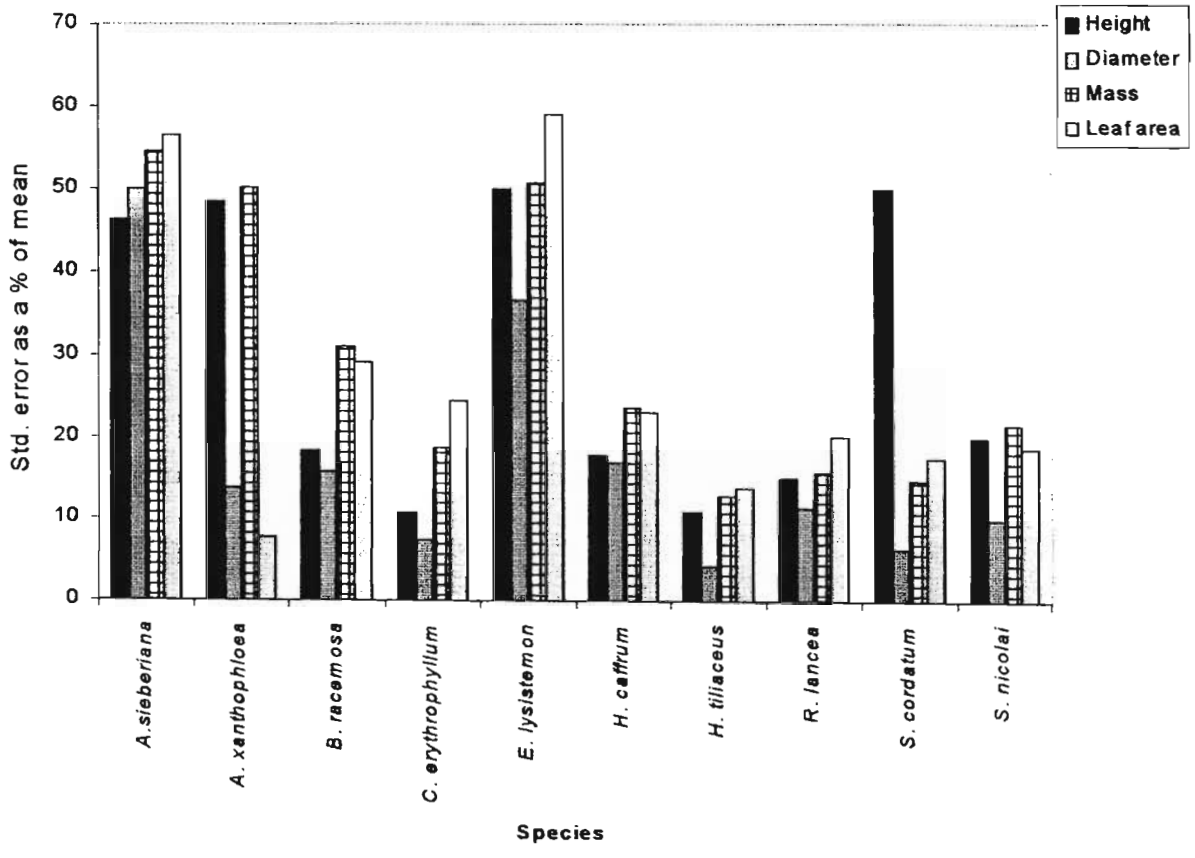


Figure 4.13: The inherent variability of each growth variable measured for each species (height and diameter growth, aboveground biomass and total leaf area), shown by expressing the standard error of the control mean of each growth variable as a percentage of that mean for each species.

Although *Acacia xanthophloea* also showed no significant difference in stem diameter growth, height growth and biomass there were also some problems with the data. There were unexplained mortalities on the control plot (Table 4.6), which when shown as zero growth in the data set resulted in low growth values (Tables 4.9, 4.10, 4.11). Thus, although growth of the species was reduced by the landfill conditions no significant difference in height growth, diameter growth as well as biomass between the control and landfill plots was found. However, by specifically focusing the comparison of growth between plots on the surviving plants only the analysis revealed that the height and diameter of *Acacia xanthophloea* as well as *Erythrina lysistemon* were reduced by the landfill conditions without topsoil (Tables 4.13 and 4.14).

The topsoil layer on the landfill slightly improved the height growth for *Erythrina lysistemon* and *Acacia xanthophloea.* It also improved the diameter growth of *Erythrina* but showed no significant improvement in diameter growth of *Acacia xanthophloea* (Tables 4.13 and 4.14). No further significant differences in *Erythrina* growth were found, however, the above ground biomass of the surviving *Acacia xanthophloea* trees was reduced by the landfill conditions and the topsoil layer resulted in no significant mass increase (Table 4.15). Furthermore, there was also a significant reduction in *Acacia xanthophloea* total leaf area on the landfill plots relative to the control and the topsoil layer appeared to provide little improvement (Table 4.12). Thus, of the species that showed no significant difference in growth in Table 4.9, 4.10, and 4.11, only the growth of *Barringtonia racemosa* was unaffected by the landfill conditions. The other species, *Acacia xanthophloea* and *Erythrina lysistemon* both showed evidence of reduced growth, whilst the variability of data for *Acacia sieberiana* was too high for reliable conclusions to be reached.

Table 4.13: Comparison of relative height increase of surviving plants between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative growth values included and dead plants removed.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	1.68 ±0.31[1] (7)[2] a[3]	1.76 ±0.68 (4) a	0.54 ±0.25 (7) a
Combretum erythrophyllum	0.56 ±0.06 (7) a	0.23 ±0.23 (5) ab	-0.24 ±0.18 (6) b
Erythrina lysistemon	0.47 ±0.22 (5) a	-0.14 ±0.10 (5) ab	-0.39 ±0.14 (4) b
Harpephyllum caffrum	1.29 ±0.23 (7) a	1.18 ±0.56 (2) a	-0.10 ±0.09 (2) a
Hibiscus tiliaceus	1.47 ±0.16 (7) a	1.46 ±0.40 (7) a	0.37 ±0.12 (7) b
Rhus lancea	0.40 ±0.06 (6) a	0.23 ±0.07 (5) a	-0.21 ±0.14 (5) b
Acacia sieberiana	0.83 ±0.49 (5) a	1.82 ±0.49 (5) a	1.17 ±0.13 (3) a
Strelitzia nicolai	0.70 ±0.14 (7) a	0.19 ±0.31 (3) a	-0.08 ±0.21 (3) a
Syzygium cordatum	-0.01 ±0.03 (7) a	-0.11±0.06 (2) a	0.06 ±0.00 (1) a
Acacia xanthophloea	2.99 ±0.43 (3) a	1.86 ±0.32 (7) ab	0.90 ±0.45 (4) b

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.14: Comparison of relative diameter increase of surviving plants between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative growth values included and dead plants removed.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	2.72 ±0.43[1] (7)[2] a[3]	2.73 ±0.90 (4) a	1.71 ±0.32 (7) a
Combretum erythrophyllum	1.91 ±0.14 (7) a	1.41 ±0.54 (5) ab	0.23 ±0.27 (6) b
Erythrina lysistemon	1.83 ±0.49 (5) a	0.60 ±0.19 (5) ab	0.19 ±0.11 (4) b
Harpephyllum caffrum	0.94 ±0.16 (7) a	0.91 ±0.07 (2) ab	-0.01 ±0.09 (2) b
Hibiscus tiliaceus	3.40 ±0.14 (7) a	2.16 ±0.49 (7) b	0.69 ±0.18 (7) c
Rhus lancea	3.41 ±0.38 (6) a	1.62 ±0.40 (5) b	0.50 ±0.25 (5) b
Acacia sieberiana	1.08 ±0.66 (5) a	0.53 ±0.18 (5) a	0.88 ±0.68 (3) a
Strelitzia nicolai	1.86 ±0.69 (7) a	0.83 ±0.52 (3) a	0.90 ±0.41 (3) a
Syzygium cordatum	1.44 ±0.09 (7) a	0.83 ±0.15 (2) b	0.17 ±0.00 (1) b
Acacia xanthophloea	9.07 ±0.48 (3) a	2.53 ±0.35 (7) b	1.81 ±0.74 (4) b

[1]Standard error of the mean.
[2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.15: Comparison of relative above ground biomass of surviving plants between the control and experimental plots i.e (biomass/ original height) after 14 months. Data presented for analysis with dead plants excluded from the data.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	4.20 ±1.3[1] (6)[2] a[3]	5.41 ±2.9 (4) a	1.01 ±0.4 (7) a
Combretum erythrophyllum	9.57 ±1.8 (7) a	7.17 ±3.5 (5) a	1.91 ±0.9 (5) a
Erythrina lysistemon	8.54 ±3.9 (5) a	1.37 ±0.6 (6) a	0.58 ±0.2 (4) a
Harpephyllum caffrum	10.59 ±2.5 (7) a	7.45 ±4.1 (2) a	0.37 ±0.1 (3) a
Hibiscus tiliaceus	54.96 ±7.0 (7) a	47.70 ±17.0 (7) a	3.63 ±1.4 (7) b
Rhus lancea	29.82 ±4.7 (5) a	8.08 ±1.9 (5) b	2.03 ±1.0 (5) b
Acacia sieberiana	20.16 ±10.2 (4) a	13.0 ±3.3 (5) a	5.90 ±2.8 (3) a
Strelitzia nicolai	6.49 ±1.4 (7) a	2.50 ±1.4 (3) a	1.32 ±0.6 (3) a
Syzygium cordatum	10.94 ±1.6 (7) a	4.13 ±0.7 (2) a	1.88 ±0.0 (1) a
Acacia xanthophloea	43.70 ±8.3 (3) a	10.60 ±2.2 (7) b	3.27 ±1.5 (4) b

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

The other six species (*Harpephyllum caffrum; Strelitzia nicolai; Syzygium cordatum; Combretum erythrophyllum; Hibiscus tiliaceus* and *Rhus lancea*) showed a more marked reduction in growth on the landfill. However, the species growth response to the additional topsoil layer on the landfill was variable.

Although the application of topsoil had a beneficial effect on growth for some species, the growth of *Harpephyllum caffrum*; *Strelitzia nicolai* and *Syzygium cordatum* was not improved. These species experienced high mortalities and the number of surviving plants on the landfill plots on which growth measurements could be made was less than or equal to 3. Therefore, primarily due to the low number of remaining replicates, analysis of the growth variables measured on surviving individuals generally resulted in no significant difference between plots ($p>0.05$). However, the analysis of the data that included dead

plants as zero clearly showed a significant reduction in species performance with or without topsoil on the landfill for *Harpephyllum caffrum*; *Strelitzia nicolai* and *Syzygium cordatum*. *Syzygium cordatum* did however have an unusually high inherent variability in height growth (50%) that resulted in no significant difference in height growth between the control and landfill plots (Figure 4.13). This was not considered indicative that growth was not affected by the landfill conditions, it was more likely that the high data variability in the data was responsible for the conclusion. However, it was clear that *Syzygium cordatum*, *Harpephyllum caffrum*, and *Strelitzia nicolai* performed poorly on the landfill and a topsoil layer was of little benefit.

Some species, such as *Hibiscus tiliaceus* and *Rhus lancea* showed a clear reduction in growth (i.e. stem diameter height and diameter, biomass, leaf area) when planted directly into the landfill cover material. However, the use of topsoil relative to no topsoil on the landfill resulted in significant ($p<0.05$) increase within all the growth variables measured for *Hibiscus tiliaceus* (Tables 4.9, 4.10, 4.11 and 4.12). *Rhus lancea* also showed a significant ($p<0.05$) increase in stem diameter and height growth on the topsoil plot relative to the no topsoil plot (Tables 4.9 and 4.10). However, unlike *Hibiscus* the topsoil layer provided no significant ($p>0.05$) improvement of tree biomass and total leaf area (Tables 4.11 and 4.12).

The topsoil layer also appeared to improve the growth of *Combretum erythrophyllum*, as there was no significant ($p>0.05$) difference in stem height growth, diameter growth, or mass between the topsoil plot and control plot. However, the improvement was minimal, as these variables on the topsoil plot were also not significant ($p>0.05$) different on the landfill plot without topsoil (Tables 4.9, 4.10 and 4.11). Interestingly, the *Combretum* leaf

area data did not differ between the control and the experimental plots (Table 4.12) and further analysis of the measured growth variables including only surviving plants showed no significant ($p>0.05$) difference in aboveground mass either (Table 4.15). It was apparent that species responded differently to the topsoil layer on the landfill, however, for *Hibiscus tiliaceus, Rhus lancea* and *Combretum erythrophyllum* the topsoil layer appeared to provide some improvement in growth.

In summary, there appeared to be a large variation in the growth response of different tree species to landfill conditions. The results indicated that *Barringtonia racemosa* growth was the least influenced by the landfill conditions. Although *Acacia sieberiana* also showed very little significant difference in growth between the plots, this was attributed to the relatively high data variability and not species tolerance to landfill conditions. The growth of *Hibiscus tiliaceus, Rhus lancea, Combretum erythrophyllum, Acacia xanthophloea* and *Erythrina lysistemon* was reduced by the landfill conditions, however, a topsoil layer over the landfill cover material helped to improve the trees growth. The growth of *Harpephyllum caffrum, Strelitzia nicolai* and *Syzygium cordatum* were significantly reduced by the landfill conditions with or without topsoil, indicating that these species were sensitive to the landfill conditions.

Although the data for some species was a difficult to interpret the following ranking of all 10 species, from least sensitive to most sensitive to the landfill environment was suggested: *Barringtonia racemosa, Combretum erythrophyllum, Acacia xanthophloea, Hibiscus tiliaceus; Rhus lancea; Erythrina lysistemon; Acacia sieberiana; Strelitzia nicolai; Syzygium cordatum* and *Harpephyllum caffrum.* The general ranking position of some species may vary slightly with the addition of topsoil, such as *Hibiscus* which should shift

up one position in the ranking because it responded relatively well to topsoil layer. Relative positions of *Erythrina lysistemon* and *Acacia sieberiana* could be questionable due to the unreliable nature of their data.

4.3.5 Species root morphology

The maximum rooting depth of the ten species after 14 months of growth, under normal conditions (i.e. the control plot) was 70cm. However, even with the use of the same topsoil type and depth the maximum rooting depth on the landfill was lower (40cm). Without topsoil on the landfill the maximum rooting depth was even shallower (20cm), this was probably due to landfill gas as well as poor soil structure (Figure 4.14). Table 4.16 shows the overall mean density of the roots per m^2 on the landfill topsoil (69.9 ±10.0 n=10) and no topsoil plots (30.6 ±4.4 n=10) were significantly ($p<0.05$) lower in comparison to the control plot (231.2 ±37.4 n=10). The lack of a significant ($p>0.05$) difference in overall root density between the landfill plots suggested that the topsoil layer did not significantly reduce the impact of the landfill environment on root density.

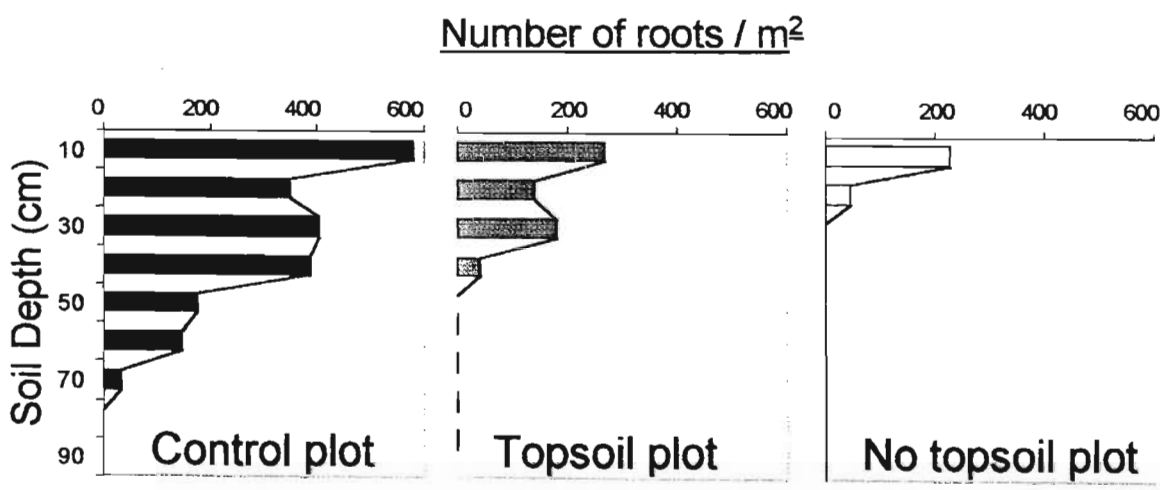


Figure 4.14:Mean root density (area) with soil depth for all species combined within each experimental plot (n=10)

Table 4.16: Total density of roots and the percentage of the total number of roots counted in the profile walls that were found in the top 10cm of the soil

	Control plot	Topsoil Plot	No topsoil plot
Mean Total density	231.2 ±37.4[1] a[2]	69.9 ±10.0 b	30.6 ±4.4 b
Mean % in top 10cm	32.7 ±6.7 a	48.4 ±6.8 a	86.1 ±4.7 b

[1]Standard error of the mean (n=10)
[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

However a significantly ($p<0.05$) lower percentage of the total number of roots recorded in each profile were found within the upper 10cm of the soil on the landfill topsoil plot relative to the landfill plot with no topsoil (Table 4.16). Considering there was no significant difference in root density between the topsoil and no topsoil landfill plots the difference in rooting depth in the no topsoil landfill plot was unlikely to be a function of reduced root growth but was an actual shallower-rooting plant response. This indicated that the topsoil layer on the landfill generally allowed for a greater proportion of roots to be deeper within the soil which could help to alleviate drought and nutrient stress usually associated with surface soil layers in a seasonal rainfall climate.

Although the data was very limited (n=1) individual species root response to the landfill with and without topsoil appeared to be variable and species specific (Table 4.17). There did not appear to be any relationship between the overall species performance and the degree to which root density was reduced by the landfill conditions. This relationship was tested by regression analysis of the percentage reduction in root density on the landfill plots relative to the control verse the above ground biomass as a percentage of the mean control biomass for each species. For the analysis *Erythrina lysistemon*, *Acacia xanthophloea*, and *Acacia sieberiana* were removed from the data set because the species

mass data was highly variable (Figure 4.13). The result of the linear regression showed no significant relationship between the reduction in root density and the mass of the species on the landfill topsoil plot ($p=0.84$; $R^2=0.009$) or no topsoil plot ($p=0.185$ $R^2=0.32$). The lack of a clear relationship between root density and differential species performance suggests that the ability to have a greater root density on the landfill was probably not the key reason for differential species performance.

Table 4.17: Total density of roots and the percentage of the total number of roots counted in the profile walls that were found in the top 10cm of the soil for each species on the three experimental plots.

Species	Control plot		Topsoil plot		No topsoil plot	
	Density	%	Density	%	Density	%
Acacia sieberiana	266.7	48.4	36.7	44.1	56.7	100.0
Acacia xanthophloea	302.2	10.9	80.0	73.5	23.3	91.3
Barringtonia racemosa	95.6	30.0	41.1	57.6	26.7	58.8
Combretum erythrophyllum	305.6	55.9	40.0	27.8	43.3	81.0
Erythrina lysistemon	365.6	60.6	37.8	32.0	25.6	100.0
Harpephyllum caffrum	423.3	24.6	80.0	16.4	27.8	92.9
Hibiscus tiliaceus	101.1	25.7	103.3	37.5	14.4	88.0
Rhus lancea	131.1	12.0	122.2	58.3	15.6	87.2
Strelitzia nicolai	115.6	55.8	55.6	86.5	23.3	62.5
Syzygium cordatum	197.8	2.8	101.1	50.5	46.7	100.0

[1]Standard error of the mean
[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

However it was noted in Table 4.17 that a large percentage of the total number of roots counted for each tree, on the no topsoil plot, were within the top 100mm of soil. The relationship between the percentage of the total number of roots in the top 10cm of soil and the above ground biomass as a % of the control mass of each species on the landfill topsoil and no topsoil plot was investigated using linear regression. As with the previous

regression analysis *Erythrina lysistemon, Acacia sieberiana* and *Acacia xanthophloea* were removed from the data set. A significant ($p=0.042$; $R^2=0.6$) negative linear relationship was found between species mass and the percentage roots in the first 10cm of soil on the landfill no topsoil plot (Figure 4.15). This suggested that the species which performed badly on the landfill no topsoil plot had a large percentage of their roots in the upper 10cm of soil whilst those which performed better were deeper rooting. This negative relationship was not clear on landfill topsoil plot and no significant linear relationship was found ($p=0.1$; $R^2=0.008$). This lack of a clear relationship would be expected as there was a significantly lower % of roots in the top 10cm of soil on the topsoil plot (Table 4.16) and the overall performance of the tree on the topsoil plot was better than the no topsoil plot.

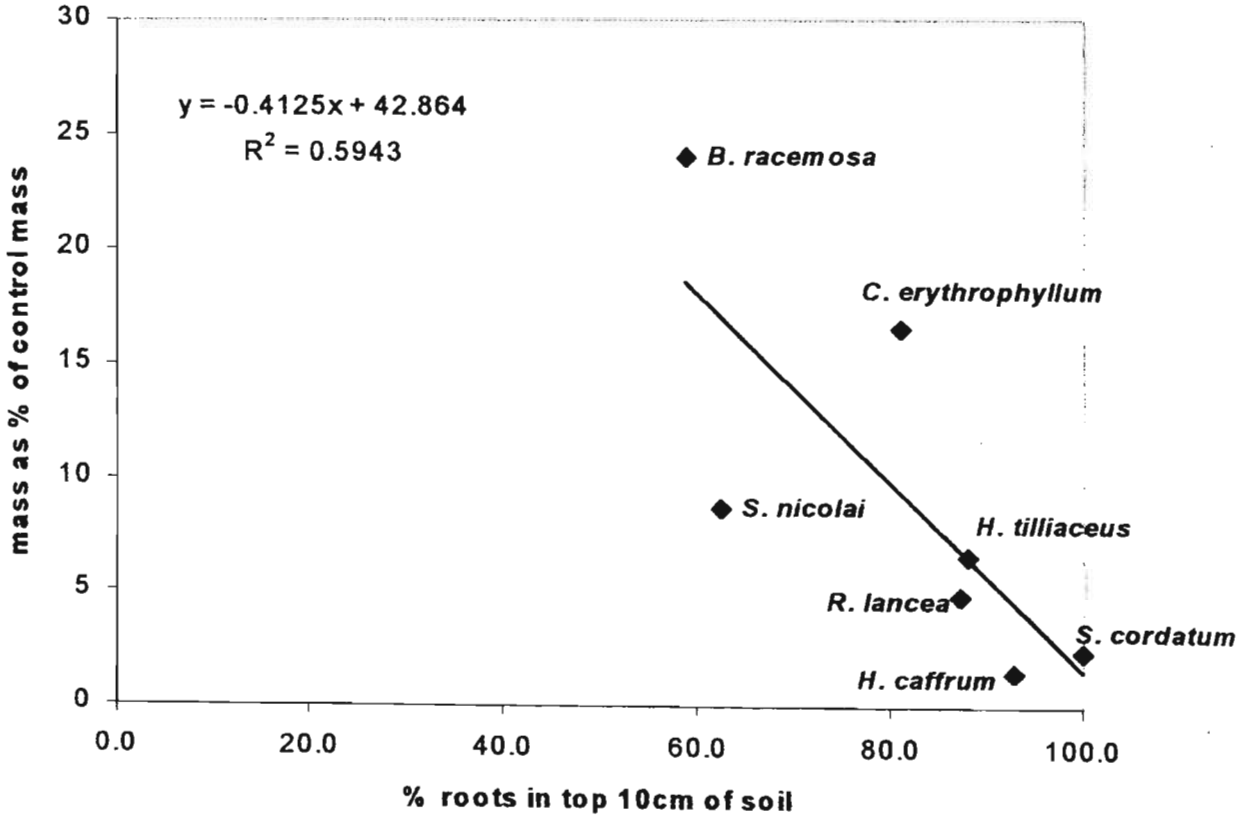


Figure 4.15: Linear regression showing the relationship between the percentage of the total number of roots in the top 10cm of soil and the above ground biomass expressed as a percentage of the control biomass for each species on the no topsoil plot.

The results suggested that for all the species the landfill conditions resulted in lower rooting densities and shallower rooting depths, however, the species which performed best on the landfill were probably able grow a greater proportion of their roots at a relatively greater depth.

4.4 DISCUSSION

The small loss of trees due to insect damage and wind can be expected in any field experiment or revegetation project. Loss of trees planted on landfills due to theft is not unique to the Bisasar Road landfill, Mackay & Richardson, (1996) reported a loss of 1.4% of 864 trees planted on Whabbs Tip, Merseyside, England. Although, the loss of trees due to theft was not large it is interesting to note that only one species, *Barringtonia racemosa*, was stolen. This species could have been targeted for resale as an ornamental plant for gardens or more likely it was taken for medicinal purposes. Extracts from *Barringtonia racemosa* plant tissues are reported to be used for making emetic solution against malaria as well as treatment of skin disease and stomach ache (Hutchings *et al*, 1996).

The overall much higher mortality of trees on the landfill by comparison to the control site confirmed the findings of many other researchers that the landfill environment presents a formidable challenge to vegetation growth, especially that of trees (Chan *et al* 1991; Lan & Wong 1994; Dobson & Moffat 1994; Ettala *et al* 1988; Flower *et al* 1981; Gill 1970; Gilman *et al* 1981; Insley & Carnell 1982; Leone *et al* 1983; Leone *et al* 1977; Moffat & Houston 1991). The reduction in the severity of the landfill environment by the application of a topsoil layer was clearly reflected in the lower mortality and improved stem growth of

some of the species. Insley and Carnell, (1982) also found that an additional layer of soil over the standard compacted cover material improved tree growth and survival.

In terms of the individual species the mortality and growth results showed that the sensitivity of plants to the landfill environment was species specific, confirming the findings of a number of other investigators from around the world (Leone *et al* 1983; Mackay & Richardson 1996; Chan *et al* 1991; Gilman *et al* 1981; Leone *et al* 1977; Flower *et al* 1981; Ettala 1988; Robinson *et al* 1992). For example, the landfill environment appeared to have no effect on the mortality and growth of *Barringtonia racemosa* irrespective of the use of topsoil. Whilst other species such as *Syzygium cordatum*, *Strelitzia nicolai* and *Harpephyllum caffrum* had very high mortalities and reduced growth with or without an additional topsoil layer. However, there were species with an intermediate response to the landfill environment such as *Combretum erythrophyllum*, *Hibiscus tiliaceus* and *Rhus lancea*. They had few or no mortalities and although growth was reduced in the trees planted directly into the landfill cover material, the use of a topsoil layer was found to ameliorate this effect. Unlike *Combretum* and *Hibiscus* there was evidence that the benefit of a topsoil layer for *Rhus lancea* was more limited.

The overall performance of the species on the landfill resulted in the following ranking from best species to worst: *Barringtonia racemosa, Combretum erythrophyllum, Acacia xanthophloea, Hibiscus tiliaceus; Rhus lancea; Erythrina lysistemon; Acacia sieberiana; Strelitzia nicolai; Syzygium cordatum* and *Harpephyllum caffrum.* The general ranking position of some species may vary slightly with the addition of topsoil, such as *Hibiscus* which should improve its ranking by one position because it responded relatively well to

topsoil layer. Relative positions of *Erythrina lysistemon* and *Acacia sieberiana* could be questionable due to the unreliable nature of their data.

It is interesting to note that almost all of the ten species used in this experiment have a normal habitat associated with rivers or water courses (Palgrave, 1984; Pooley, 1994). However, two of the species which performed best, namely *Barringtonia racemosa* and *Hibiscus tiliaceus* are usually found fringing swamps or tidal lagoons (Palgrave, 1984; Pooley, 1994). Therefore, the normal habitat of these trees would often be flooded, resulting in anaerobic soil conditions similar to those found on the landfill, thus, possibly explaining the better performance of these species in the experiment. A similar association was found by Arthur *et al* (1981) between flooding-tolerant species and tolerance to landfill gases. Therefore, the flood-tolerant characteristics of tree species are worthy of further investigation for the selection of species suitable for landfill revegetation.

The root morphology data also provided some insight into the differential species performance on the landfill. It was apparent that the landfill with or without a topsoil layer reduced the density of tree roots. Gilman *et al* (1981) also noted reduced tree root growth in landfill field investigations, which was correlated with high levels of soil CO_2 and low soil O_2. The effect of reduced root growth in landfill soils caused by elevated soil CO_2 and low soil O_2 was further confirmed by laboratory based experiments conducted by Marchiol *et al* (2000).

A shallower rooting response in landfill environments is not an uncommon phenomenon (Chan *et al* 1991; Gilman *et al* 1982; Gilman *et al* 1981). It has been suggested that those species which could direct their root growth towards the surface and away from the landfill

gas source are more successful in growth on landfills (Gilman *et al* 1982; Gilman *et al* 1981). It has also been noted that trees with a shallower root system are more susceptible to water stress in landfill soils, which usually have poor structure (Chan *et al* 1991). However, it has been suggested that species, which are normally shallow rooted, should be relatively tolerant of the low soil moisture conditions and their shallow-rooting behaviour would reduce the exposure to landfill gas found at higher concentrations deeper in the soil (Gilman, 1989).

In this experiment the opposite trend was apparent. Although all species showed a change in root growth towards the surface, it was apparent that those species that could maintain a greater proportion of their total number of roots deeper within the soil were more successful (Figure 4.15). This would suggest that the avoidance of the low soil moisture levels usually associated with the surface soil layers combined with the ability to continue deeper rooting into soils which have high concentrations of landfill gas may be a better strategy resulting in greater survival and growth of trees on landfills. The ability to maintain a greater rooting depth and avoid the soil moisture deficit in the surface soil layer was also identified as beneficial for species survival on landfills by Liang *et al* (1999). However, the work by Liang addressed soil compaction as the key factor that was limiting rooting depth and not landfill gas. Nonetheless shallower rooting which was previously considered beneficial for survival and growth on landfills may be detrimental to plant survival in climates with prolonged seasonal dry periods.

The results indicated that plant rooting depth was controlled by the soil gas composition confirming the work of Chan *et al* (1991), Gilman *et al* (1982) and Gilman *et al* (1981). By comparison of the topsoil plot and the no topsoil plot rooting depth appeared to be limited

at similar concentrations of landfill gas. The soil depth at which methane was 53-57%, CO_2 20-27% and O_2 1-2% was the common limit at which roots would no longer penetrate. These CO_2 and O_2 concentrations were comparable with those found by Gilman *et al* (1981) who noted that roots stopped growing or were redirected towards the surface when CO_2 was 8-23% and O_2 was 4-18%.

It has been noted since as early as 1946 that the effect of high carbon dioxide concentrations in the soil atmosphere varies greatly between plant species (Flower *et al* 1981; Geisler, 1963; Leonard & Pinkard, 1946; Stolwijk & Thimann, 1957). It is important that the relationship between soil gases in landfill soils is clearly understood. For example, although there is no evidence to show that methane is phytotoxic it does play a significant role by reducing the concentrations of oxygen by displacement and by the bacterial oxidation of methane into carbon dioxide. Thus, it may contribute to the increasing of concentrations of the more toxic carbon dioxide (Chan *et al* 1991; Flower *et al* 1981, Leone *et al* 1977) and to the lowering of soil oxygen (Flower *et al* 1981).

In this study there was a significant negative linear relationship between methane and oxygen, and between carbon dioxide and oxygen in the soil atmosphere. One explanation for this would be that methane and carbon dioxide displaced oxygen (Chan *et al* 1991, Lan & Wong, 1994, Moffat & Houston, 1991). As in this investigation Chan *et al* (1991) and Lan and Wong, (1994) found a significant positive correlation between methane and carbon dioxide. However, here methane was only detected in the soil when carbon dioxide concentrations were in excess of about 9% and oxygen levels were already depleted to below 7%. The raised carbon dioxide level and reduced oxygen level showed that landfill gas was infiltrating into the soil atmosphere, so therefore one would expect methane to be

detected. This suggests that when oxygen concentrations are high any methane infiltrating into the soil atmosphere is completely oxidised by soil bacteria into carbon dioxide, and so methane is not detected, and carbon dioxide levels increase. However, when oxygen levels are depleted to below about 7%, by this process of oxidation (and displacement by carbon dioxide), there is no longer sufficient oxygen to oxidise all the methane, and therefore, methane concentrations in the surface soil atmosphere become detectable. Therefore these results provide evidence that bacterial oxidation of methane in the landfill soils is also responsible for the consumption of oxygen and thus the lower levels of the oxygen measured (Haarstad, 1997; Hoeks, 1983).

Table 4.18 shows the range of landfill root zone methane, carbon dioxide and oxygen concentrations reported, from field studies, to be responsible for poor plant growth and survival. Methane concentrations ranged between 0.9%- 50% whilst carbon dioxide and oxygen concentrations ranged between 1% - 21%, and 4.7% - 17.8%, respectively. Differential species tolerance and specific additional varying landfill soil conditions (e.g. bulk density, moisture, nutrients etc.) affecting plant susceptibility to landfill gas, may be responsible for the varying results between the different field studies.

The summary of gas concentrations reported to be responsible for poor vegetation growth, indicate that if the soil methane, carbon dioxide and oxygen concentrations, are in excess of 14%, 14% and less than 12%, respectively, then high plant mortality and stunted growth can be expected (Table 4.18). Considering that the carbon dioxide, methane and oxygen concentrations in the cover material on the Bisasar Road landfill (i.e. in the no topsoil plot) were 48.4%, 41.9% and 0.6% respectively, the resultant overall poor tree survival was to be expected.

Table 4.18: Summary of the percentage methane, carbon dioxide, and oxygen in the soil atmosphere in field studies, in decreasing order of methane concentrations, and their reported effects on plants.

Measurements made in the field				
CH_4	CO_2	O_2	Reported effects.	Reference
50	21	12	[a] Landfill in Battle Creek, Michigan: Dead Red Pine.	Leone *et al* 1977
39-45	----	----	[c] Coalgate Landfill: No vegetation. Well vegetated area had CH_4 of 0-5%.	Wong, 1988
22	15	11.5	[a] South Coast Botanic Gardens, Los Angeles: Built on an old landfill. Dead *Cytisis racemosis*.	Leone *et al* 1977
16.64	9.8	10.3	[b] Gin Drinkers Bay Landfill: Strong correlation between plant cover and high landfill gas concentrations.	Wong & Yu, 1989
16.1	17.6	9.7	[b] Gin Drinkers Bay Landfill: 10 different tree species experienced inferior growth and high mortality.	Chan *et al* 1991
14.0	----	4.7	[c] Pitsea landfill: Very low survival of five tree species.	Moffat & Houston, 1991
10.3	15.1	12.7	[b] Gin Drinkers Bay: Harmfull effects on trees, shrubs and climbing plants found. Lack of O_2 and high CH_4 and CO_2 correlated with poor vegetation cover.	Lan & Wong, 1994
10.3	15.1	12.7	[b] Gin Drinkers Bay Landfill: Much lower soil fauna and vegetation growth.	Wong *et al* 1992
6-8	----	----	[c]Sefton Meadows Landfill: No vegetation. Well vegetated area had CH_4 of 0-3%.	Wong, 1988
6.1	14.7	16.1	[a]Edgeboro Landfill: Roots of Green Ash stayed near surface. Green Ash tolerant to low oxygen environments.	Gilman *et al* 1982
5	18	16	[a]Edgeboro Landfill, New Jersey, America. *Fraxinus lanceolata* avoided low oxygen soil conditions by producing adventitious roots. Hybrid poplar re-directed root growth towards the soil surface, however did not survive as well.	Gilman *et al* 1982
5	----	----	[c]Cross Lane Landfill: No vegetation.	Wong, 1988
4.8	12.8	12.1	[a]Edgeboro Landfill: Hybrid poplar saplings died after 3 years, Green ash was stunted but still alive. Roots remained close to surface.	Gilman *et al* 1982
4.8	10	14	[c] Whabbs Tip, Merseyside, United Kingdom. Poor growth of trees was attributed to no single factor, rather a combination of landfill gas and poor soil conditions.	Mackay & Richardson, 1996
0.9	5.5	17.8	[a]Edgeboro Landfill: Trees on the landfill had higher mortality and poorer growth, however this was attributed to the combination of landfill gas, soil temperature, bulk density and moisture.	Gilman *et al* 1981
---	>15	10-12	[b] Gin Drinkers Bay Landfill: Inhibited most plants.	Chan *et al* 1991
----	1-10	----	[a]Edgeboro Landfill: American Basswood growth was affected by relatively low conc. of CO_2.	Gilman *et al* 1981
---	---	<10	Root growth inhibited.	Flower *et al* 1981

Site Location [a] United States of America; [b] Hong Kong; [c] United Kingdom

The much higher concentrations of methane and carbon dioxide measured on the Bisasar Road landfill by comparison to the concentrations measured on other landfills, as summarised in Table 4.18, could be related to the depth of decomposing waste material. The area of the investigation on Bisasar Road landfill had approximately 30m of decomposing waste beneath it, whilst landfills with relatively lower soil gas concentrations had a shallow depth of waste. For example, the Pitsea landfill had maximum depth of 7m (Moffat & Houston, 1991), Edgeboro landfill had 9m of waste (Gilman *et al* 1981), and the Cross Lane landfill had only 3m of waste fill (Wong, 1988), all of which had methane and carbon dioxide concentrations within the range 1 – 18%, which were considerably less than the concentrations measured on the Bisasar Road landfill. Coalgate Lane Landfill had a comparable depth of waste (20m) with the Bisasar Road Landfill, and also had a comparable levels of methane (39-45%) in the soil atmosphere (CO_2 and O_2 were not measured) (Wong, 1988). This reinforced the suggestion of a positive relationship between the depth of the waste and higher landfill gas levels in the soil atmosphere.

Considering the depth of the waste in a landfill site can influence the concentrations of landfill gas in the soil, it would probably also influence the success of vegetation growth. However, the installation of a passive or active gas venting system could help to reduce the concentrations of methane and carbon dioxide in the soil. For example, the Gin Drinkers' Bay Landfill had a greater depth of waste (57m) than the Bisasar Road landfill, however, it had a passive gas venting system installed, resulting in lower maximum methane (17%) and carbon dioxide (18%) concentration measured (Lan & Wong 1994).

Several laboratory investigations used gas concentrations comparable with those on the Bisasar Road landfill. Arthur *et al* (1981) conducted a laboratory experiment in which one

year old *Acer rubrum* (red maple) and *Acer saccharum* (sugar maple) were planted in soil fumigated with 3% O_2, 40% CO_2, 50% CH_4 and 7% N_2 for 48 days. Both species suffered chlorosis and abscission of the lower leaves. Another laboratory experiment conducted by Leonard and Pinckard, (1946) using cotton seedlings found that with oxygen concentrations maintained at 21%, a carbon dioxide concentration of 30-45% reduced root and shoot growth, whilst a carbon dioxide concentration of 60% prevented all root growth and greatly reduced shoot growth. This showed that even under high ambient oxygen concentrations high carbon dioxide levels can effect plant growth. It may be concluded that carbon dioxide in landfill soils has a more important role in determining root growth than oxygen concentration (Chan *et al* 1991). Considering the high level of carbon dioxide found in the root zone of trees on the Bisasar Road landfill the resultant high mortality and reduced growth seen in this experiment was probably primarily due to the soil CO_2 conditions.

The application of a topsoil layer over the original landfill cover material, resulted in a significant reduction in the concentrations of methane and carbon dioxide in the soil atmosphere, but had no significant effect on the low levels of oxygen. Muntoni & Cossu, (1997) found similar results in which a layer of compost reduced landfill gas emissions. In this study, the regression analysis of oxygen versus carbon dioxide (R^2=0.53; $p<0.01$) showed that oxygen in the soil atmosphere was reduced to zero when carbon dioxide concentrations were in excess of 21%. This measured reduction in oxygen with increasing carbon dioxide was probably due to displacement by carbon dioxide and methane and methane oxidation (Barry, 1987; Chan, *et al* 1991; De Rome *et al*, 1997; Dobson & Moffat, 1994). Although the concentrations of carbon dioxide and methane were significantly reduced by the application of topsoil, the levels, 25.6 (±0.7) and 22.3 (±1.3),

respectively, were still sufficiently high to result in very low oxygen conditions, and no significant difference in soil oxygen between the two landfill plots was measured. The levels of carbon dioxide and methane in the topsoil on the landfill were still in excess of concentrations found to be generally associated with high plant mortality and poor growth (Table 4.18).

The ratio of carbon dioxide to methane in the topsoil layer was not significantly different to that in the landfill cover material. Therefore, it is unlikely that the bacterial oxidation of methane into carbon dioxide was taking place at a faster rate in the topsoil layer in comparison to the landfill cover material. If this is the case, then the bacterial oxidation of methane did not account for the significantly reduced concentrations of methane measured in the topsoil plot. The increased physical resistance to the flow of landfill gas presented by the topsoil may have played a part in reducing gas concentrations. Landfill gases tend to flow along paths of least resistance from the decomposing waste to the atmosphere (Flower *et al* 1981). The layer of topsoil could probably, either slow the flow of gas, or change the main direction of flow, thus, reducing the concentrations of methane and carbon dioxide detected in the topsoil layer. Another possible explanation may be that the lower compaction of the topsoil layer in comparison to the landfill cover material resulted in a greater influx of gases from the atmosphere, thus diluting the concentrations of gases in the soil atmosphere. However, neither of these ideas were tested in this present investigation.

The soil temperature on the landfill plots was significantly higher than that on the control and was positively correlated with soil methane and carbon dioxide levels. The exothermic decomposition of waste produces warm gases which can warm the soil as it filters through, therefore the raised soil temperature and correlation with landfill gas concentrations are not

uncommon (Chan *et al* 1991; Gilman *et al* 1981). However, similarly to the finding of Moffat and Houston (1991) the thicker layer of cover material provided by the additional topsoil layer (topsoil plot) resulted in lower soil temperature. This was attributed to the greater distance between the surface soil layers and the underlying heat source (decomposing waste), as well as the significantly lower carbon dioxide and methane levels that were found in the topsoil layer. However the soil temperatures measured on the landfill and the control were within the optimum range for tree growth of 10-30°C (Ruark *et al* 1982), therefore it was unlikely to be a factor resulting in differential tree survival and growth.

From the soil chemical and physical analysis of the conditions on the control and experimental plots K, Mg, pH, conductivity, extractable Mn, soil moisture and stone content were significantly different between the plots. Therefore, of the soil chemical and physical characteristics measured these were the most likely to be responsible for any differences in tree performance between the plots.

The concentrations of K (168.7 mg kg^{-1}) were significantly higher in the landfill cover material (no topsoil plot) by comparison to the topsoil (topsoil plot and control plot). However, deficiencies, and not high concentrations, of K are more likely to cause poor plant growth and survival (Munshower, 1994). Thus, the high concentrations of K in the landfill cover material would probably not have any negative effect on tree performance. Mg concentrations were significantly lower in the landfill cover material by comparison to the topsoil (topsoil plot and control plot). Again, the main concern about Mg is soil deficiencies and not excesses (Munshower, 1994). The soil Mg concentrations (168.3 mg kg^{-1}) in the landfill cover material were within the 'normal' soil range of 40 – 5000 mg kg^{-}

[1] (Grimshaw *et al* 1989), although the concentrations were in the lower part of the 'normal' range.

Moffat and Bending, (1992) and McKendry, (1996) recommended similar soil pH ranges suitable for revegetation of 3.5 – 8.5 and 4.5 – 8, respectively. Therefore, although the pH of the landfill cover material (pH 8.1) was significantly higher than that of the soil used on the topsoil plot and control plot (7.2 - 7.4) it is unlikely to have accounted for the differences in the tree performance observed. However, it is interesting to note that higher soil pH values are often associated with anaerobic conditions since the reduction process removes hydrogen ions from the soil solution thus the pH of acid soils on landfills can rise considerably (Smith, *et al* 1999).

The minimum standard of soil conductivity for woodland establishment on landfills is <2mS cm^{-1} (Moffat and Bending, 1992), however, the conductivity in the landfill cover material (no topsoil plot) was 3.7mS cm^{-1} in this investigation. This higher conductivity in the landfill cover material is often caused by leachate contamination and can have a negative influence on vegetation performance (Dobson & Moffat, 1994; Tong & Wong, 1984). The raised pH and the significantly higher concentration of K in the cover material, as found by Winant *et al,* (1981) in leachate irrigated soils, reinforced the evidence of leachate contamination of the landfill cover material (no topsoil plot). This indicated that further leachate related variables, not measured in this investigation, such as depressed soil solution osmotic potential (Cureton *et al* 1991), increased sulphate, sodium, chloride and metals in the soil (Ettala, 1988), which can have negative effects on vegetation, may have influenced the trees growth and survival. The conductivity of the topsoil placed on the

1989; Menser *et al* 1979). However, the reducing conditions of anaerobic soils result in the highly soluble manganous ion Mn^{2+} (Crawford, 1989; Menser, *et al* 1979; Munshower 1994). Therefore, the anaerobic soil conditions on the landfill plots probably resulted in the formation of the highly soluble Mn^{2+}. This is of significance as high Mn concentrations can be phytotoxic to many sensitive species. High available manganese concentrations induce iron chlorosis, and also cause brown necrotic spots on plant leaves, due to antagonism between these ions for uptake by the roots (Crawford, 1989). Mn is not toxic if the soil pH is greater than 5.5 because the manganese solubility is reduced with increasing soil pH (Munshower, 1994; Winant *et al* 1981). Therefore, it may be argued that in a soil with a pH value of 7.4 (topsoil plot) and 8.1 (no topsoil plot) available Mn concentrations should be very low. However, the anaerobic soil conditions on the landfill plots may have formed strong reducing conditions. Thus, landfill soils may be a relatively unique situation with high pH and strongly reducing conditions. However, it must be noted that the sampling of the soil would have removed the reducing conditions, however, the measured extractable Mn levels were still very much higher. This would suggest that the anaerobic conditions changed the ratio between total and extractable Mn, which was maintained even after the anaerobic conditions had been removed (i.e.on air drying the sampled soil before analysis). It can be concluded that the high level of available Mn in the topsoil and cover material of the landfill, in conjunction with the anaerobic conditions may have influenced the growth and survival of the trees.

The landfill cover material had significantly lower soil moisture, as found by Gilman *et al* (1981) in a similar experiment. Highly compacted soils usually have reduced soil hydraulic conductivity and volumetric water content (Ruark *et al* 1982; Taylor & Brar 1991), possibly explaining the lower moisture content in the landfill cover material, which are

characteristically compacted (Dobson & Moffat, 1994). The application of topsoil over the cover material on the landfill, and so increasing the soil depth, significantly improved the moisture levels on the landfill, as found by Moffat & Houston, (1991). However, the moisture levels in the topsoil plot were still significantly lower than the control plot. Considering that species tolerant to flooded soils (i.e. soils with high water content) have shown tolerance to high landfill gas conditions (Arthur *et al,* 1981), the low moisture conditions on the landfill may present a problem for such plant species.

Considering that all the plots were sufficiently close to each other as to receive the same rainfall, a possible explanation for moisture differences may be the physical structure of the waste cover material. The structure of the landfill cover material below the topsoil on the landfill may prevent the upward migration of soil moisture into the topsoil during dry periods and reduce infiltration of rain water into the plot without topsoil, thus possibly accounting for the different moisture conditions. A possible explanation for the higher moisture levels in the control plot may be the underlying weathered dolerite silty clay. This may allow for the upward migration of moisture during dry periods, unlike the cover material on the landfill. The significant difference ($p<0.01$) in stone content on the landfill plot by comparison to those receiving topsoil may help to confirm the suggestion that landfill soil structure reduces upward capillary movement of water. Thus, depending on the size of the stones, the continuity of the soil pore space maybe lacking, thus preventing upward migration of moisture by capillary action. Similarly, the 'cementing' together of the stones by the increased compaction possibly results in decreased rainfall infiltration.

The stone content of soil is measured in different ways by different researchers. It is measured by volume, and / or weight and the size of the particles defined as stones differs

considerably. This makes it difficult to compare these results with other research conducted. However, it can be concluded that lower stone contents are usually preferred for landfill restoration (McKendry 1996; Moffat & Bending 1992). Therefore, the application of topsoil with a significantly ($p<0.01$) lower stone content, over the landfill cover material, should have resulted in improved conditions for tree root growth.

In summary the key environmental variables in the landfill cover material which were most likely to influence the growth and survival of the trees would have been: the high carbon dioxide, and low oxygen concentrations in the soil atmosphere, high conductivity, high extractable Mn concentrations, low soil moisture and high stone content and possibly low magnesium concentrations. The application of topsoil was able to reduce the severity of carbon dioxide concentrations, although it still remained within the range of concentrations at which poor plant growth and survival could be expected. The topsoil also had a better moisture content, stone content, conductivity and level of Mg. However, the Mn concentrations were just as high as the landfill cover material suggesting that topsoil quality can deteriorate with time when used on landfills.

CHAPTER 5: TREE GROWTH AND SURVIVAL: A SOIL FUMIGATION EXPERIMENT

5.1 INTRODUCTION

The field-based research has provided information about the range of environmental conditions on the landfill and the varying response of different tree species (Chapters 3 & 4). However, the heterogeneity and dynamic nature of the landfill environment and the high mortality of less 'tolerant' species made it difficult to identify key plant characteristics, which could explain differential species performance on the landfill. It was also difficult to establish the relative importance of high CO_2 and low O_2 concentrations in the soil and the potential for antagonistic, additive or synergistic effects between these two variables.

To provide an experimental approach, a soil fumigation system was designed, constructed and established. Using bottled gas, the experimental apparatus was capable of mixing carbon dioxide and oxygen into four different ratios, thus supplying four different gas regimes. A combination of automated low pressure mixing of gases and a pulse flow fumigation technique made the experimental apparatus uniquely economical to operate, thus allowing for relatively longer fumigation periods to be achieved. The apparatus was used to fumigate the soil of 80 potted landfill 'tolerant' (*Barringtonia racemosa*) and 'non-tolerant' (*Harpephyllum caffrum*) trees. The 4 gas regimes (treatments) used were the following: "normal" soil O_2 and CO_2; high CO_2 and normal O_2; low O_2 and normal CO_2; high CO_2 and low O_2.

Using the fumigation system, the hypothesis that one species (*Barringtonia racemosa*) was significantly more tolerant than the other (*Harpephyllum caffrum*) was tested. Measurements were made of the above and below ground growth and functional plant morphological, anatomical, and physiological characteristics to evaluate the responses of the two species to the 4 soil gas treatments.

5.2 THE SOIL FUMIGATION SYSTEM

5.2.1 Design objectives

A fumigation system consists of an apparatus, which provides gases at known concentrations to chambers in which plants can be exposed. The usual fumigation system provides gases into the chamber which change the gas mixture of the atmosphere around plant shoots with the plants rooted in pots of soil. A soil fumigation system must change the gas concentrations in the soil atmosphere, thus the chambers are containers in which plant roots and not shoots are exposed. Most fumigation experiments have focused on atmospheric gases and relatively little research has been done on the soil atmosphere and its effect on plants. The major focus of most soil atmosphere research has been on the effects of waterlogging on plants and the effects of low soil oxygen and high carbon dioxide. Most researchers, such as Bacanamwo and Purcell (1999); He, *et al* (1999), and Loreti and Osterheld (1996) induce low oxygen conditions by the actual flooding of the soil. However, this technique does not allow for the easy manipulation of the actual oxygen or carbon dioxide concentrations i.e. to predetermined or desired levels. In order to achieve this some researches such as Huang *et al* (1997), Moog and Bruggemann (1998), and Voesenek *et al* (1999) preferred using hydroponic solutions that could be flushed with the required concentration of oxygen, carbon dioxide and nitrogen. However, these experimental methods do not create an experimental environment common to the usually

low oxygen, high carbon dioxide, and *dry* soil of a landfill. Therefore, although similarities in plant responses to waterlogging and landfill conditions have been observed by Arthur (1981), Barry (1987) and Chan *et al* (1991), and waterlogging research provides insight into potential plant responses, none of the experimental systems used and described to date could be directly adapted to the needs of this experiment.

Of primary design importance for this experiment was complete control of the gas concentrations in the fumigation chambers otherwise the system would not be an improvement relative to field experiments. Secondly, in order to assess the response and adaptation of relatively large and slow growing tree saplings, the system had to fumigate large volumes of soil for a long period of time. Based on the conclusions from the field experiment (Chapter 4) the within species variability and duration before health affects were observed, an experimental period of 140 days and at least 10 replicates per species per treatment were deemed necessary. Each tree would require its own chamber so as to increase the validity of the replication, especially to avoid pseudoreplication, thus a total of 80 identical chambers were required (2 species x 4 treatments x 10 replicates).

Research relating directly to the effects of landfill gas on plants has been mostly field-based and suffers the usual high level of environmental heterogeneity which often makes it difficult to draw definitive conclusions. There have been relatively few studies that involve the simulation of landfill soil gas conditions. Chan *et al* (1991; 1998) described a fumigation system that used simulated landfill gas generated through the anaerobic digestion of pig manure. The largest of their experiments consisted of a single simulated landfill gas treatment with 10 replicate pots in which the roots of 10 species of tree were fumigated for 42 days. The gas diffused from the bottom of the pot through the soil and

flowed freely from the soil surface into the surrounding atmosphere. The gas concentrations measured within the pots were highly variable and there was no direct control of the gas composition. Due to the free flow of gas from the soil surface into the atmosphere, the contamination of the air around the shoots with the simulated landfill gas, could also make the interpretation of plant response to root fumigation difficult. The experimental period was only one third of the duration required for this experiment and the reliability of anaerobic digesters as a gas supply was a concern. The use of a single pot / fumigation chamber for 10 trees also raised concern for the validity of the replication and if root responses of individual plants could be measured. Thus the fumigation system described by Chan *et al* (1991) was not suitable for the needs of this experiment.

The fumigation system used by Arthur *et al* (1981) was very similar to Chan *et al* (1991), but used only two 88 L garbage cans in which the roots of two species of maple were fumigated for 50 days. Unfortunately, this design also lacked the scale and level of replication needed for this experiment. However, it did make use of a cylinder of pre-mixed gas instead of anaerobic digestion as a simulated landfill gas supply. However, the gas concentrations within the soil still showed high levels of fluctuation even with a more reliable gas supply. Arthur *et al* attributed the high level of fluctuation to the variability in atmospheric conditions. This highlighted the need for control of the atmospheric influence on the soil gas concentrations, and suggested that the free flow of gas from the soil surface into the surrounding atmosphere was probably not a suitable approach.

Marchiol *et al* (1999) described a 12-day experiment assessing seed germination of 4 different ground covers in three replicate atmospheres of simulated landfill gas. The fumigation chambers were sealed boxes with gas flow output pipes that prevented over

pressurisation but allowed for a positive gas pressure to be maintained within the chamber. This reduced possible atmospheric influence on the gas concentrations in the chamber. The system also made use of bottled gas that was commercially pre-mixed to the desired oxygen and carbon dioxide concentrations. The result was better control of gas concentrations within the chambers. The use of a pre-mixed gas and a slightly pressurised chamber was a suitable solution for control of gas composition. However, the scale of Marchiol's design was significantly smaller in comparison to the needs of this experiment, and when scaled-up the cost of premixed gas could be prohibitive, further, the chamber design was not suitable for the fumigation of tree roots. Marchiol *et al* did use an interesting device for the splitting of the gas supply into equal gas flows for each of his 12 chambers. The device operated using two simple principles. The first was the total diameter of the gas output pipes should be less than the diameter of the gas distribution cylinder, and the second was that sufficient gas, in terms of pressure, was available in the distribution cylinder to supply all the chambers (*pers com* Marchiol, 1999). However, the design would need marked adaptation in order to handle 80, much larger, chambers instead of 12.

The work by Zhang *et al* (1995) also made some interesting design contributions. They were interested in the fumigation of tree roots with simulated landfill gas for investigating the response of nitrogen fixing root nodules on two leguminous species. Zhang *et al* made use of bottled gas but used separate cylinders of nitrogen, carbon dioxide and oxygen and mixed the gases by setting the flow rates of the three component gases according to the treatment requirements. This was a more economical way of achieving complete control of the gas concentrations than commercially premixed gas. Again the scale of the experiment was relatively small, with only 32 chambers that were fumigated for 8 days. There were

some further interesting design features that were worth noting. The roots of the trees were fumigated by placing the pots into plastic bags, with inlet and outlet pipes attached, and the bag was sealed around the base of the stem ensuring fumigation of the root material only. The gas outlet from the bag was a small diameter pipe that was submerged in water, thus allowing for a positive pressure to develop inside the bag and preventing the influence of external atmospheric conditions. Although, the use of a plastic bag is probably not robust enough for an experiment of 140 days the idea of using a semi-closed fumigation system appeared to be a sensible design feature. Zhang *et al* (1995) also used an interesting technique for ensuring equal distribution of gases between the 8 pots within each treatment. This was done by fumigating each pot in sequence, controlled by a timer and eight solenoid valves. Although this was an innovative approach the cost of a solenoid valve for every chamber was regarded as too expensive.

This review of the other landfill gas fumigation experiments provided some interesting design features that have been mentioned. However, in terms of the aims of this experiment as well as the number of treatments, number of trees and the duration needed for this experiment, it appears to be unique and no single fumigation apparatus designed to date fits the exact requirements. Thus in order to achieve the goals of this experiment a combination of the ideas used in previous fumigation systems and some new ideas were required. These are discussed in the next section.

In terms of the fumigation treatments, it is generally agreed that the direct effect of soil methane on plants is minimal relative to low oxygen and high carbon dioxide concentrations (Dobson & Moffat, 1994; Leone *et al* 1977; Spreull & Cullum, 1987). In this investigation, the chapter on grasses concluded that elevated carbon dioxide levels in

the soil were the greatest factor limiting grass growth and methane had very little direct effect (Chapter 2). A similar conclusion was made by Chan *et al* (1991) and Wong and Yu (1989) who observed a greater negative correlation between soil carbon dioxide concentrations and vegetation cover relative to that of methane. Methane appears to have no direct toxicity to plants and concentrations as high as 60% have been shown to have no phytotoxic effect (Flower, *et al* 1981). In fact methane cannot be metabolised by plants and has been used as a tracer gas in transpiration studies (Morris & Dacey, 1984). Thus, the experimental focus taken in here was on the effects of oxygen and carbon dioxide only and used inert nitrogen as a carrier gas.

In order to get a detailed assessment of the plants response to the treatments it was important that the plants were growing and not killed by too severe treatment conditions. Using the gas-depth profile in conjunction with rooting depth and tree mortality from the previous field work and a literature survey of measured landfill soil gas concentrations (Table 3.10), a carbon dioxide concentration of 25% and oxygen concentration of 3% were considered suitable thresholds for the fumigation experiment. In order to investigate the effects of these gases the following 4 treatments and mixed soil gas concentrations were used: normal soil O_2 (20%) and normal CO_2 (2%); high CO_2 (25%) and normal O_2 (20%); low O_2 (3%) and normal CO_2 (2%); high CO_2 (25%) and low O_2 (3%).

5.2.2 Design

A schematic diagram of the fumigation system design is provided in Figure 5.1 with the details and the reasons for particular design choices provided below. Gases supplied in high-pressure cylinders are a common source used for fumigation systems, however, it is more expensive than gas produced through anaerobic digestion, but the quality is far more reliable. The use of a commercially premixed gas regime in a single cylinder is convenient and ensures accurate control of the gas concentrations, however, for an experiment of this size and duration the cost of premixing was prohibitive. Separate gas cylinders of gas that can be used to mix the required gas regimes at low pressure was a more economical approach. Technical grade carbon dioxide, oxygen and nitrogen were purchased from Afrox (Pty Ltd) and supplied separately in the largest available high-pressure cylinders, 31.3kg (15300kpa), 11.5kg (17500kpa) and 11kg (20000kpa), respectively. In order to prevent system failure due to lack of gas, a number of cylinders for each gas were connected by a high-pressure manifold. The manifolds for the three gases were constructed out of appropriate cylinder adapters and Parflex 27579kpa pressure rated flexible piping, and connected 5 cylinders of nitrogen, 4 carbon dioxide and 2 oxygen into three separate manifolds (Figure 5.2 and 5.3).

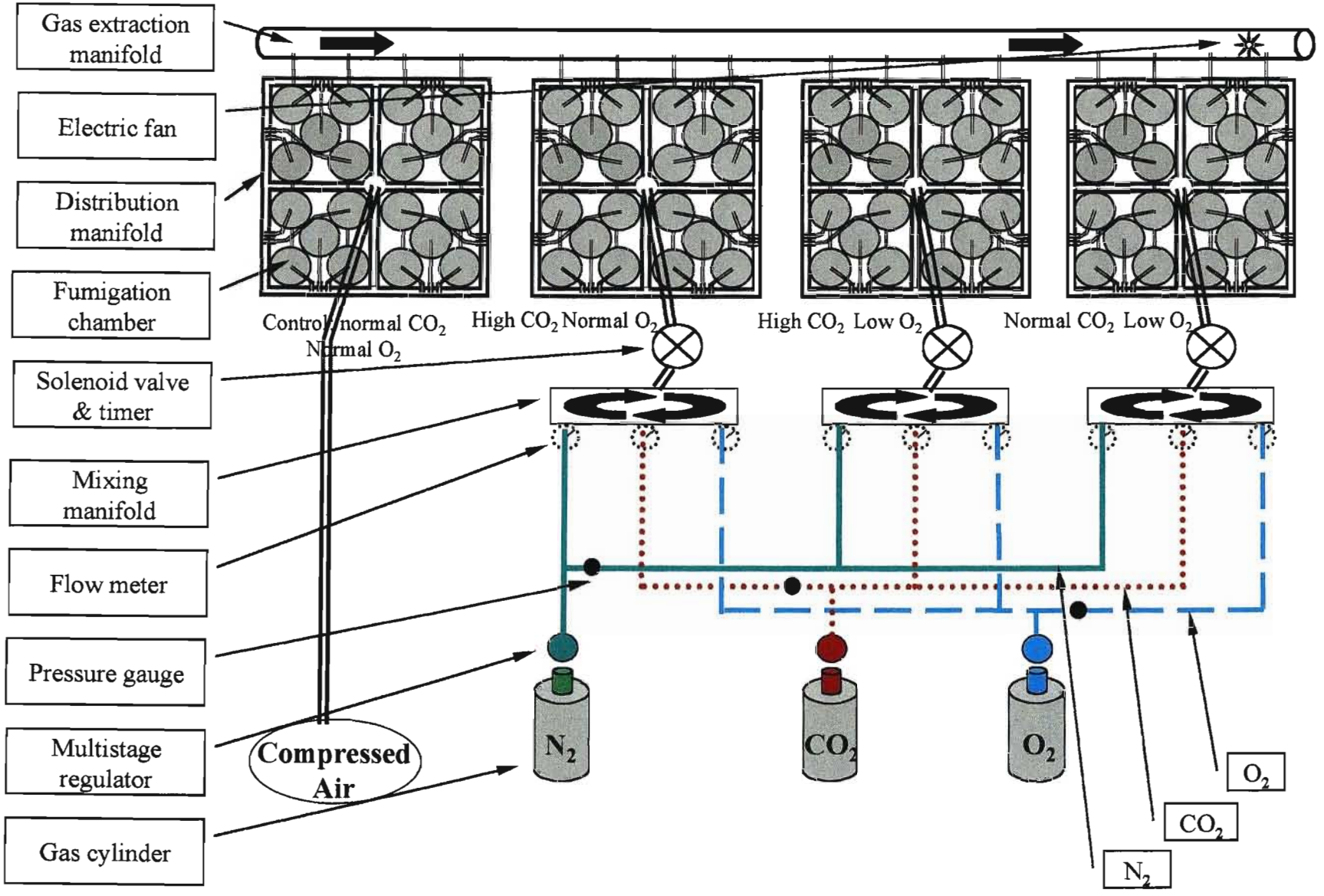


Figure 5.1 :Schematic diagram of soil fumigation system

To achieve the desired soil atmosphere conditions for the control, compressed air supplied by an electric compressor was more economical than commercially supplied cylinders of compressed air. The air supply from the compressor was passed through an oil trap, to prevent contamination, and the pressure regulated to 200kpa.

Three separate multistage regulators, also adjusted to a constant output pressure of 200kpa, controlled the gas supply, from the high-pressure manifolds, needed for the gas treatments. The gas from each regulator was connected with 6mm poly-natural nylon pressure tubing and divided, using 6mm brass elbows and T-junctions, into 3 separate gas outlets to be used for each treatment. It was critical for the mixing of the gases that there was sufficient volume of gas within the poly-natural tubing to supply the demand of the three treatments at the same time and without a pressure gradient developing. The 200kpa supply pressure was calculated as sufficient, however, a set of pressure gauges were installed in-line between the regulators and the mixing manifolds to monitor pressure conditions within the supply line during operation (Figure 5.4).

The mixing of the gases into the appropriate gas ratios was achieved using Dwyer Visi-Float flow meters and specially designed gas-mixing manifolds. Each treatment required a set of three flow meters and a mixing manifold to achieve the required gas ratios (Figure 5.5). The control did not require any special mixing of gases, thus the air supply was connected directly to a single flow meter without a mixing manifold. In order to achieve the desired gas ratios it was important to note that the actual quantity of gas (molecules) measured using a flow meter varies with the specific gravity (SG) and the pressure of the gas used. Thus it was important to convert the observed flow meter reading into an actual gas flow which was pressure and SG corrected using the following equations.

Pressure correction

$$Q_2 = Q_1 \times \sqrt{\frac{P_2}{P_1}}$$

Q_1 = Observed flow meter reading

Q_2 = Actual flow corrected for pressure

P_1 = Standard atmospheric pressure, 14.7 PSI

P_2= Actual Pressure, 14.7 PSI + pressure inside flow meter

Specific gravity correction

$$S_2 = S_1 \times \sqrt{\frac{1}{S.G.}}$$

S_1 = Observed flow meter reading

S_2 = Actual flow corrected for specific gravity

1 = Specific gravity of air

S.G. = Specific gravity of gas being used in flow meter originally calibrated for air

Using the above equations the flow meters were adjusted to provide the required ratio of each gas flowing into the mixing manifolds. The manifold was a thick wall PVC chamber 100mm in diameter and 500mm long (4 l liquid volume) with the appropriate fittings for the flow meters and the outflow of the mixed gas attached by plastic welding (Figure 5.6). Although the operating pressure of the gas entering the chamber was 200kpa the chamber was designed for a pressure of at least 300kpa, so as to ensure safety.

A 220 Volt solenoid valve on the outflow of the mixing manifold controlled the flow of the three gases through the flow meters (Figure 5.7). When the valve was closed the mixing manifold would fill up to a pressure of 200kpa with the correct ratio of the three gases, as controlled by the flow meters. The opening of the solenoid valve released a pulse of 200kpa mixed gas into the gas distribution manifold and started the cycle of mixing a new batch of gas within the mixing manifold. The opening and closing of the solenoid valves for each treatment was synchronised and automatically controlled by a series of electronic relays connected to a timer (Figure 5.8).

The reason for using a pulse gas flow rather than a continual flow was the first step to dealing with one of the largest design challenges, achieving equal gas distribution between the twenty separate chambers within each treatment. It was critical to have sufficient pressure within the distribution manifold so as to provide all the chambers with equal volumes of gas without a pressure gradient in the manifold developing. However, maintaining a high pressure in an open system is wasteful of gas and costly, and using low pressure is more economical but results in poor distribution. Thus, the use of a pulse flow system allowed a relatively high pressure to be maintained during pulses, thus improving gas distribution but minimising gas wastage. However, the design of the distribution manifold was also critical in terms of reducing the volumes of gas required.

The distribution manifold needed to be small in volume and have 20 small outlets so, even as an open system, it could be easily pressurised by a relatively high volume pulse from the mixing manifold. A simple linear distribution manifold with the inlet on one side proved to be inefficient and required higher than 200kpa to ensure good distribution. This lead to the experimentation with a number of different designs and the distribution was assessed by

the pressure required to attain even distribution. The best approach was a manifold constructed from 16mm diameter PVC piping (Figure 5.9). A single supply pipe was divided into 4 branches that were connected to the four quarters of a continuous ring of 16mm pipe onto which the outlets were evenly spaced. This design ensured that any single outlet would receive gas from two directions simultaneously, thus reducing the development of a pressure gradient within the manifold even at a relatively low pulse pressure of 200kpa. The distribution manifold was also designed as 35% of the total volume of the mixing manifold and the outlet diameters were restricted to 4mm diameter thus ensuring good pressure development within the manifold with each pulse even though it was an open system.

The distribution manifold outlets were fitted with 4mm push-clip pneumatic hose attachments (FESTO, Pty Ltd), although expensive they proved to be very useful because they allowed for easy attachment and detachment of chambers without having to worry about gas leaks. The fumigation chamber feed pipes were made of thick wall 6mm poly-natural nylon tubing that plugged directly into the FESTO clips. It was critical that the feed pipes were all the same lengths and had no kinks, ensuring equal gas resistance and even gas distribution.

The fumigation chambers were made of 20 l polypropylene buckets with lids that seal (Figure 5.10). Gases did not diffuse directly from the surface of the soil into the atmosphere but were ducted away from the foliage of the plants. The chamber was closed off around the stem of the trees, ensuring only the fumigation of the roots. Also this was important for creating back pressure within the chambers allowing better fumigation of the soil, better gas distribution between chambers and better gas use efficiency. It was also

important that the atmosphere surrounding the plants was not contaminated with the fumigation gases as this could effect plant response and create potentially hazardous working conditions. The tree stem was inserted through a 50mm diameter hole in the centre of each lid, using a split cut through half the lid so as not to damage the foliage. Once the lid was placed onto the chamber the split and hole surrounding the stem were sealed using silicon and Genkem foam sealant. These products were found to have sufficient flex so as not to restrict stem growth but still maintained a good seal.

A 60mm screw cap was inserted into the lid of the chambers providing easy access for watering and monitoring the condition of the soil. The gas inlet, gas sampler and gas outlet, which were made of poly-natural tubing, were inserted into the chamber through three separate 6mm holes in the lid (Figure 5.10). The gas inlet tube was glued to the inside of the chamber and the gas was diffused by a 50mm cylindrical air-stone attached to the centre of the base of the chamber. The bottom 5cm of each chamber was filled with 7mm stone to assist with even gas distribution and water drainage in the soil. A polypropylene tap was plastic welded onto the base of each chamber allowing for excess water to be drained if needed. The gas sampler consisted of a 50mm air-stone that was placed into the centre of the potting medium just below the root ball of each tree. After passing through the lid, the sampler tube was sealed with a 6mm plastic plug, which could be removed when a gas sample from the chamber was drawn. The gas outlet was fixed just below the lid of the chamber and allowed for the flow of the gas from the chamber into the gas removal manifold.

Equal lengths of poly-natural tubing were used to remove the gas from the top of the chambers into a 110mm diameter, 7m long PVC pipe. The PVC pipe was fitted with a

small electric fan so as to maintain a very slight negative pressure within the manifold preventing any back flow of atmospheric air into the system. The manifold transported the waste gas out of the greenhouse in which the experiment was conducted.

The fumigation system was in a fully air-conditioned greenhouse with temperature and humidity control. The day / night temperature was controlled to one degree accuracy at 24°C and 20°C respectively, and the relative humidity was kept constant at 50%. As a precautionary measure a temperature-activated alarm was designed and installed, which provided a warning if the temperature varied more than 5°C from the target value. Minimum and maximum temperatures within the greenhouse was checked daily so as to ensure that the air-conditioning system was working properly and the desired conditions were maintained.

Gaseous carbon dioxide is a 'denser-than-air' asphyxiant. A concentration of 10% (100 000ppm) can produce unconsciousness and death, lower concentrations can cause headaches, sweating, rapid breathing, mental depression, visual disturbance and shaking (Mallinger, 1996). Large quantities of bottled carbon dioxide were present within the greenhouse and although an exhaust gas removal system was operational, a precautionary gas leak warning system was important. An electric air pump positioned on the floor of the greenhouse continuously circulated greenhouse air through a clear container of bromothymol blue solution. This blue indicator solution turns yellow in the presence of carbonic acid, which would form if the air bubbling through contained elevated levels of CO_2. The solution could be seen from outside the greenhouse, thus providing a clear

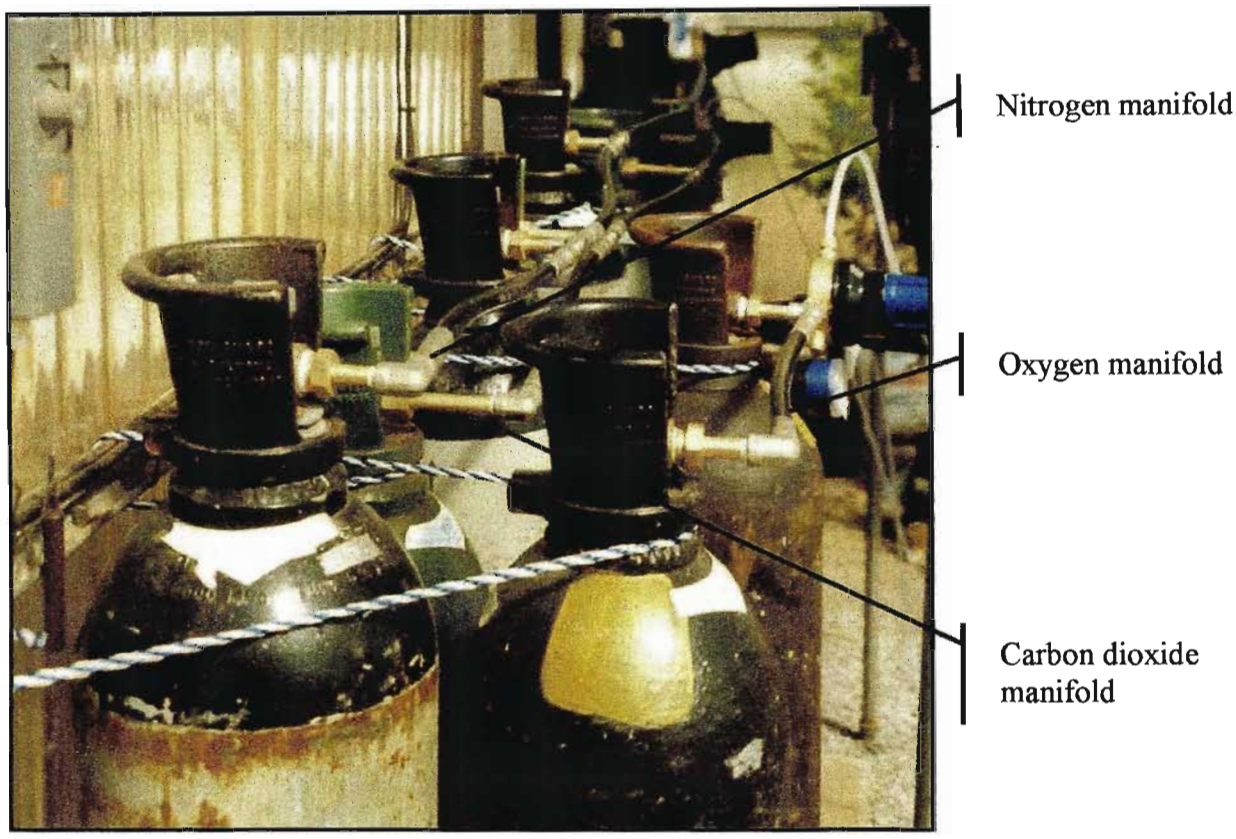


Figure 5.2: A set of cylinders of nitrogen, oxygen or carbon dioxide were connected by a high pressure manifold so as to supply a reliable gas source for the fumigation system.

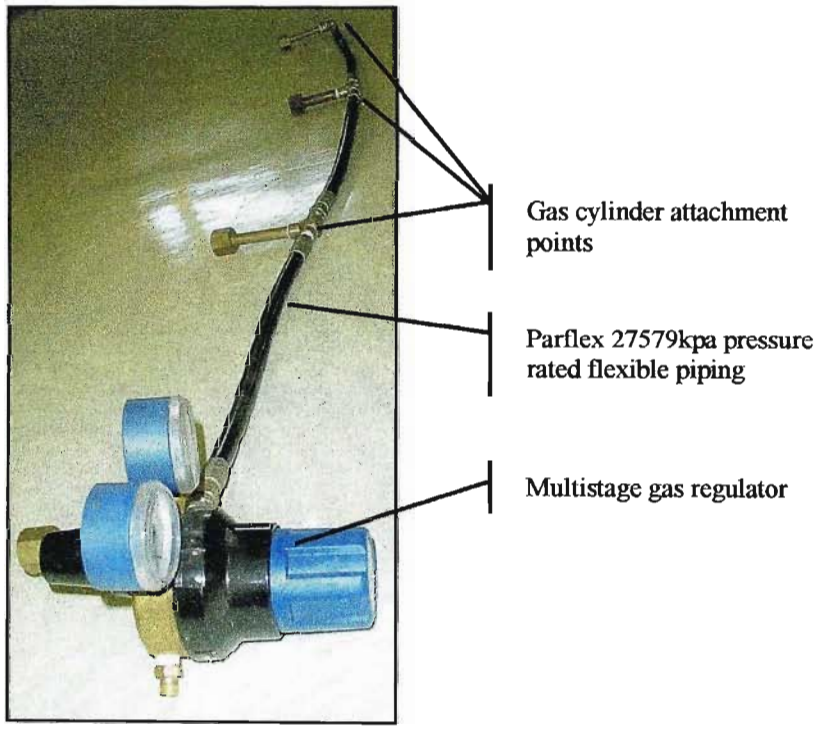


Figure 5.3: Example of a high-pressure manifold disconnected from the cylinders, showing multistage regulator and gas cylinder attachment points.

Figure 5.4: In-line pressure gauges installed in the N_2, CO_2 and O_2 gas supply tubes in order to ensure a 200Kpa supply pressure to the mixing manifold was maintained.

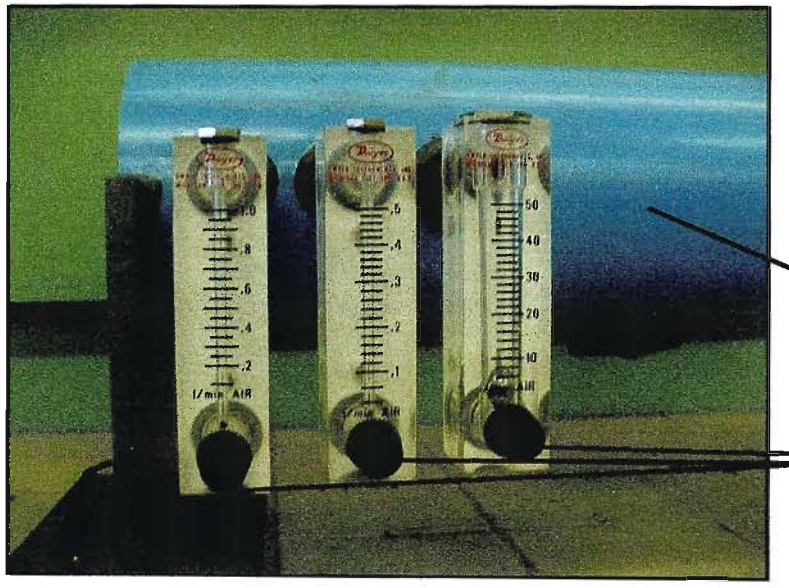


Figure 5.5: The ratio of N2 ; CO2 and O2 in the mixing manifold for each treatment was controlled by three separate flow meters.

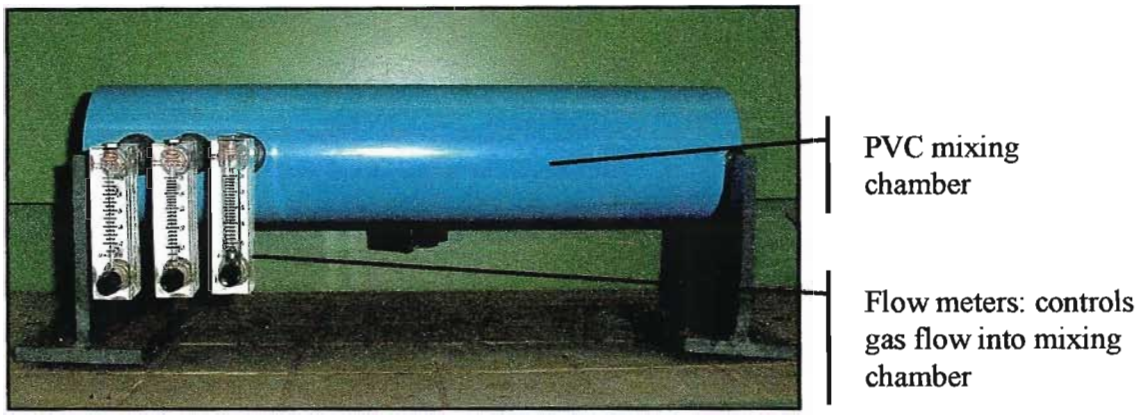


Figure 5.6: Mixing manifold made of thick wall PVC which filled up with the three gases in the appropriate ratio for the treatment as controlled by the flow meters.

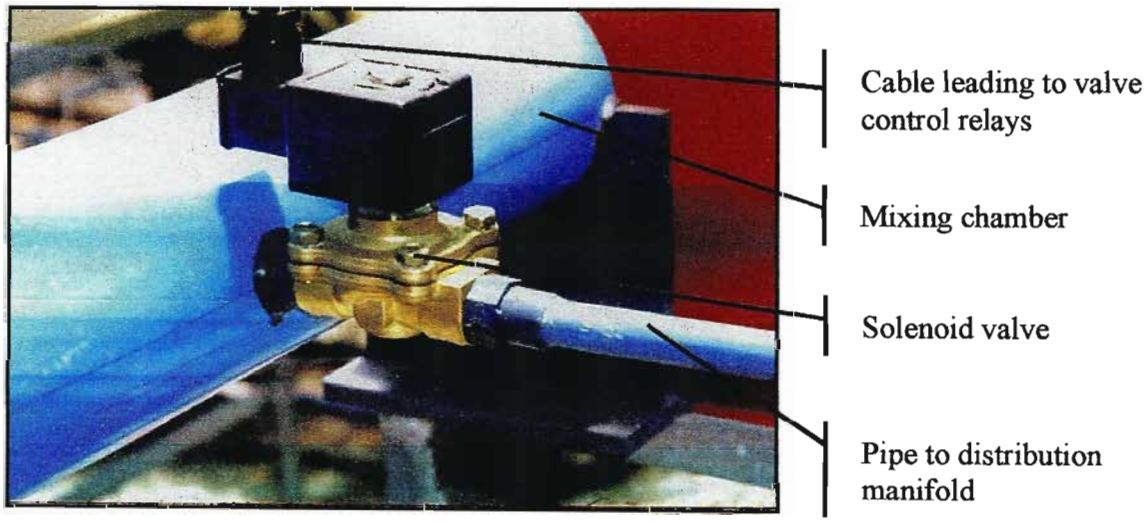


Figure 5.7: Solenoid valve on the outflow of the mixing manifold allows for a pulse gas flow to the distribution manifold to be achieved.

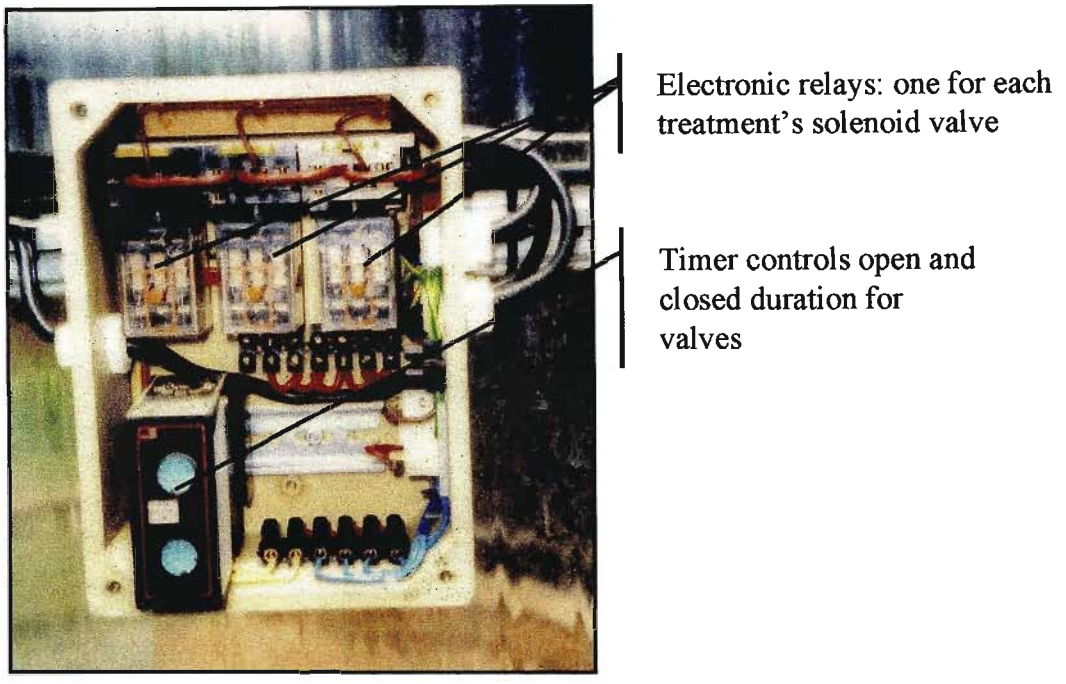


Figure 5.8: Electronic relays and timer used to control the synchronised opening and closing of the solenoid valves for each treatment.

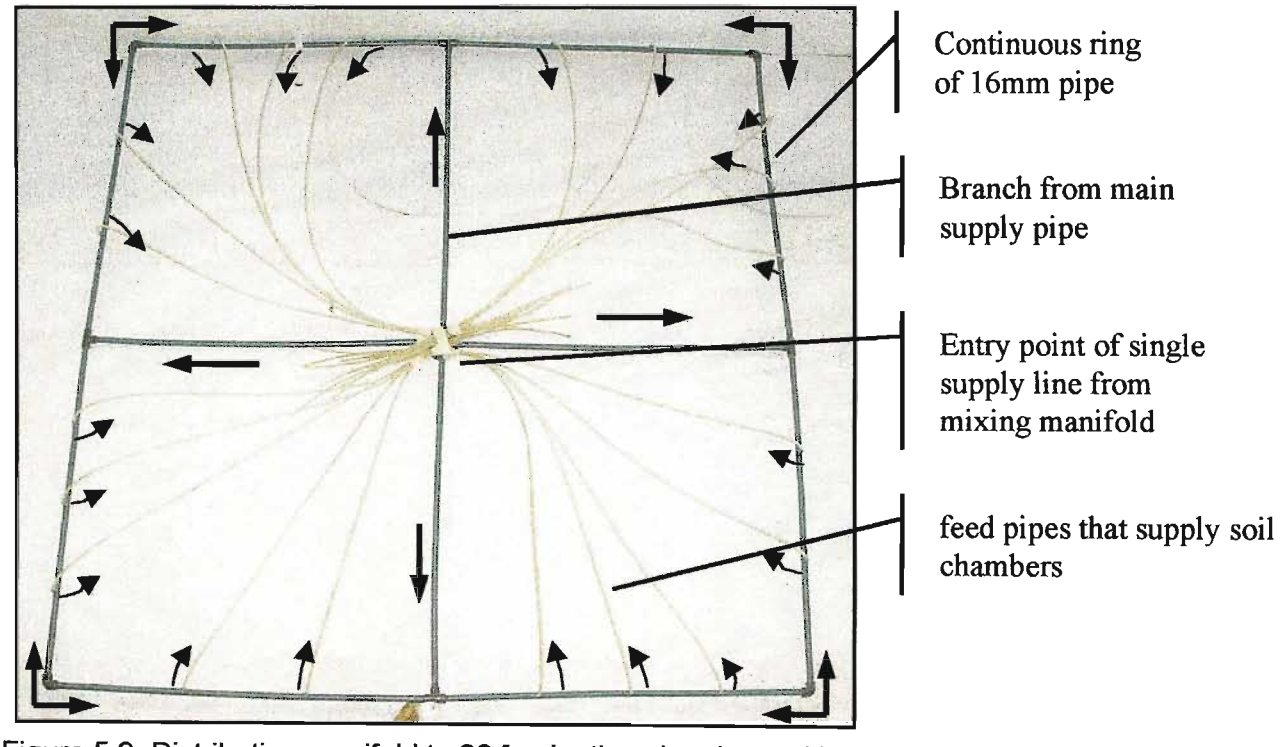


Figure 5.9: Distribution manifold to 20 fumigation chambers with arrows showing direction of gas flow

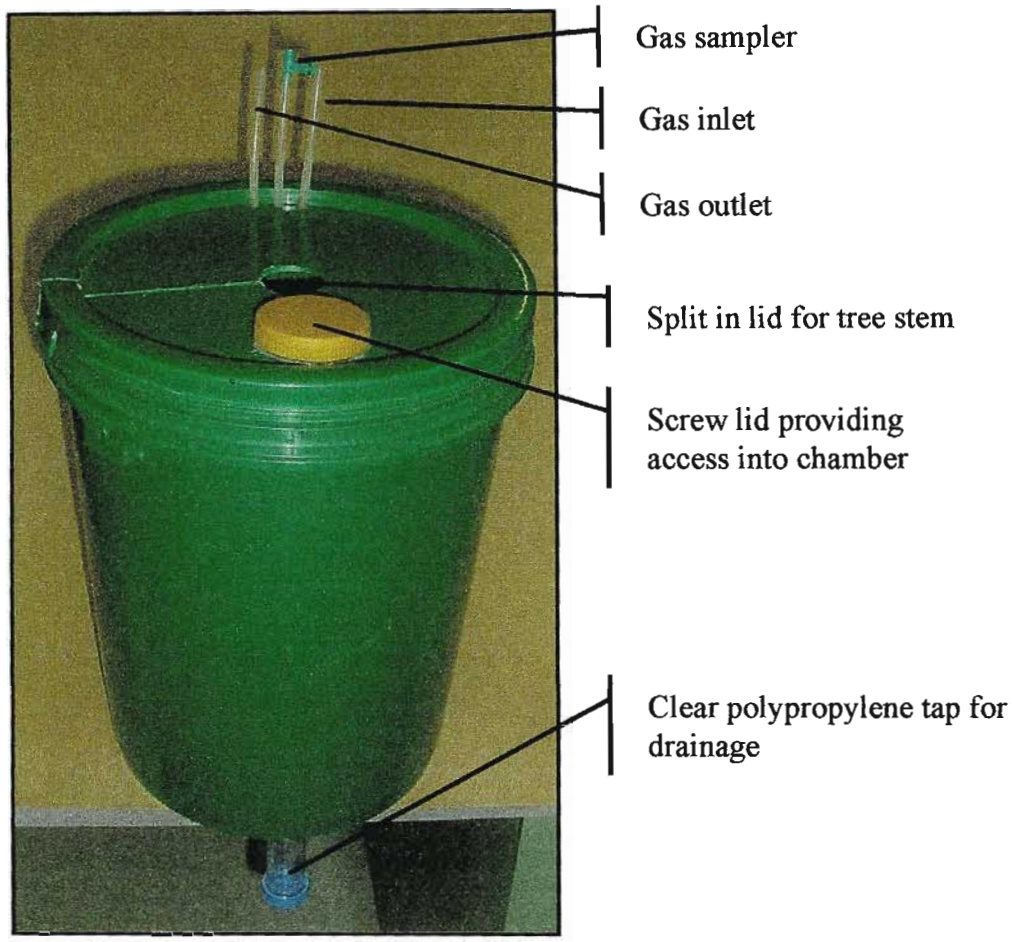


Figure 5.10: Fumigation chamber constructed from a 20l polypropylene bucket

indication of a CO_2 hazard before entering. The levels of oxygen and carbon dioxide within the greenhouse were also checked daily for the first week and then weekly for the rest of the experimental duration using an infra red gas analyser (Geotechnical Instruments GA 94 Infra– Red Gas Analyser).

5.2.3 System evaluation

The flow meters were set according to the theoretical flow rates required to achieve the different gas treatments and the mixed gas flow was checked using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. During calibration the actual concentrations of CO_2 and O_2 after mixing did not vary more than 3% from the theoretical values calculated from the flow meters, illustrating the efficiency of the mixing manifold.

Initially the solenoid valves were kept open until the air in the chambers was displaced, the valves were then closed and the concentration of gases in the soil within the chambers was carefully monitored. This procedure was done 10 times to calculate the valve closed time and open time that was optimal for maintaining a consistent gas concentration in the chambers without gas wastage. This was subsequently set to 20 minutes closed, 18 seconds open.

The gas concentration within each chamber was monitored 4 times a day during the first 3 days to ensure stability of the gas flow. Thereafter the gas concentration within each chamber was measured every 7 days, using the infra– red gas analyser, to ensure the system was working satisfactorily (n=22). The desired soil atmosphere treatments were achieved (Figure 5.11). The 'high' values, 'low' values or the 'normal' values between the

treatments for either of the individual gases, carbon dioxide and oxygen, were not significantly ($p>0.05$) different. However, the 'normal' values were significantly ($p<0.05$) different from the 'low' and 'high' values within and between the treatments.

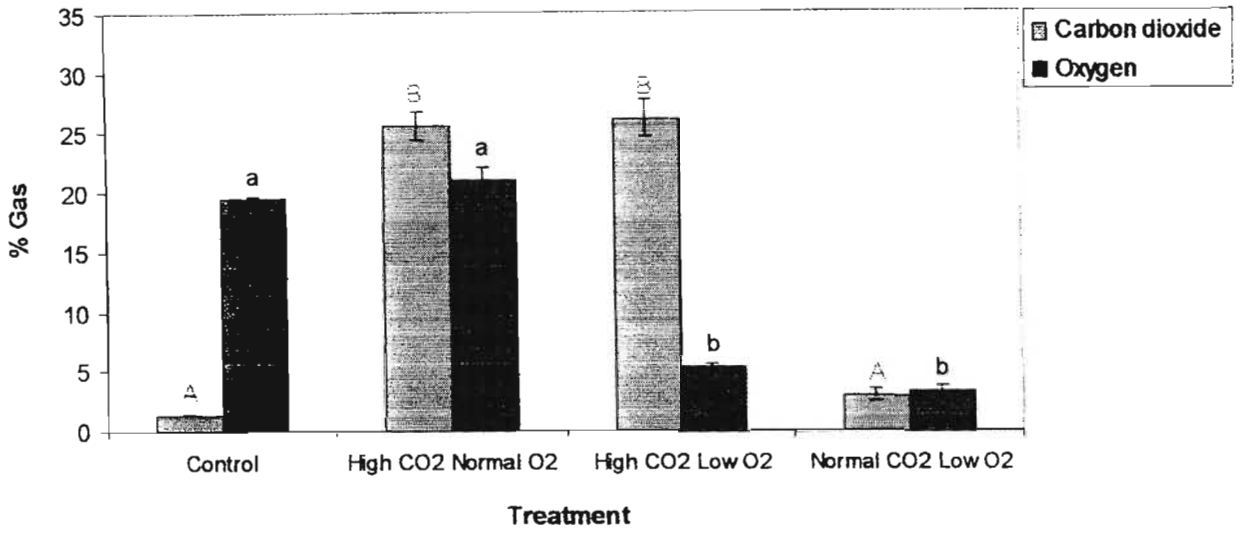


Figure 5.11: Mean (n=22) percentage carbon dioxide and oxygen in the soil of the chambers for each treatment. Significant differences ($p<0.001$) between gas levels between treatments are shown by a change in letters (upper case letters for CO_2, lower case for O_2).

Relatively small variations in the gas concentrations were measured between the chambers within each treatment (Table 5.1), indicating a satisfactory distribution of the mixed gas between the 20 chambers. The waste gas extraction system also worked satisfactorily, as no increase in carbon dioxide levels in the greenhouse were detected and levels remained at approximately 394 $\mu mol\ mol^{-1}$ throughout the experiment, as measured with a LI-COR Portable Photosynthesis System -LI-6400.

Table 5.1: The mean and the 95 percent confidence limit of carbon dioxide and oxygen concentrations measured between the chambers within the treatments during the experimental period (20 chambers per treatment measured 22 times).

Treatment	Carbon dioxide		Oxygen	
	Mean[1]	95% confidence limit	Mean	95% confidence limit
Control	1.38	0.28	19.53	0.27
High CO_2 Low O_2	26.40	1.56	5.17	0.94
High CO_2 Norm. O_2	25.56	1.14	20.97	0.23
Norm. CO_2 Low O_2	2.94	0.08	3.30	0.38

[1]Mean (n=22) calculated from the means for each treatment (n=20).

In terms of gas use efficiency the nitrogen, oxygen and carbon dioxide cylinders in the high-pressure manifolds only needed filling every 20, 31 and 70 days respectively. Thus a total of 35 nitrogen, 9 oxygen and 8 carbon dioxide cylinders were used to fumigate 80 chambers for the 140 day experiment. The fumigation system achieved its design objectives and the overall efficiency of the system and ease of use makes it suitable not only for this experiment but provides for further research opportunities using different plant species and gas fumigation regimes. The system can be used for relatively rapid primary screening of plant species for suitability for landfill revegetation and further research into the chronic effects of plants to different soil gas mixtures.

5.1 MATERIALS AND METHODS

5.3.1 Plant materials and treatment

One year old saplings of *Barringtonia racemosa* and *Harpephyllum caffrum* were supplied from the local nursery. The saplings of each species were carefully chosen so as to be of similar height and condition. They were supplied in 500ml plastic potting bags containing

standard potting soil (90% pine bark). In order to differentiate between old roots and new roots, the root balls, with the original potting soil, were lightly teased and placed into a nylon net bag (6mm square mesh). The trees were planted into the 20*l* fumigation chambers uniformly packed with a 1:1 sieved topsoil: washed river sand mixture. A river sand topsoil mixture was used so as to ensure even gas distribution during fumigation and easier removal of the soil medium from the roots at the end of the experiment. So as to ensure successful transplantation, the condition of the trees was monitored for a month before the chambers were closed and fumigation began. A Kelway 16-F soil moisture meter was used for weekly checks on soil moisture within each fumigation pot and moisture levels were kept constant by the addition of an appropriate amount of water.

There were twenty chambers per treatment with 10 replicate trees per species. The trees were fumigated for 140 days from the 8th of January 2001 to 27 June 2001 with the following treatments: "normal" soil O_2 (20%) and CO_2 (2%); high CO_2 (25%) and normal O_2 (20%); low O_2 (3%) and normal CO_2 (2%); high CO_2 (25%) and low O_2 (3%). Unfortunately the treatments could not be randomly positioned within the greenhouse as the distance of the chambers, within each treatment, from the distribution manifolds needed to be equal to ensure equal gas distribution. However, the greenhouse was relatively small and measurements of light intensity and air temperature showed no significant differences ($p>0.05$) between the areas in which the chambers for each treatment were positioned.

5.3.2 Measurement of above ground structure and development

The stem diameter of the trees, measured with digital callipers, was calculated from the mean of two diameter measurements taken perpendicular to each other at 5cm from the point of entry into the fumigation chamber for each stem. Stem height was measured from the point of stem entry into the fumigation chamber to the tip of the tallest apical shoot. The increase in stem diameter and height of each tree was determined by subtracting the original tree size from that of subsequent measurements. The number of leaves on each tree at the beginning and end of the experiment as well as any leaf loss during the experiment was also recorded. The overall condition of the plants was observed on a daily basis and any stress responses, such as epinastic curvature of the leaves or wilt were recorded. The final oven dry mass (105°C) of the above ground plant material was determined at the end of the experiment. Dry stem and leaf mass were determined separately so as to provide information about plant resource allocation. A sample of three leaves of similar age, which had developed during the experimental period, was taken from a similar position on 10 trees of each species within each treatment. The leaf area was measured using a CI 251 Leaf area meter (CID, Inc. NW Camas, Washington, U.S.A.), and dry mass was used to calculate leaf area mass ratios.

5.3.3 Physiological measurements

Stomatal conductance, A-Ci response curves (assimilation rate plotted against intercellular CO_2 concentration), and light response curves were measured using an open gas exchange system (LI-COR Portable Photosynthesis System -LI-6400, Li-Cor Inc., Lincoln, U.S.A.). Stomatal conductance was measured on a single fully mature leaf in the second whorl of the plant from each treatment for both species (n=10). Each set of measurements was completed within a morning and repeated approximately every 2 weeks during the experimental period. Measurements were taken only on sunny days and required 4 hours to

complete all 80 plants. Therefore, sampling had to be done in order to compensate for any possible changes in illumination due to movement of the sun or clouds. This was done by taking readings from only one plant per species in each treatment at one time. After all the treatments had been sampled in this manner, measurements were repeated in a similar way until all 80 plants had been sampled (Arthur, *et al* 1981). Other than the flow rate within the measuring chamber, the environmental conditions were not controlled and the stomatal conductance was measured as soon as ΔH_2O stabilised to less than 1%.

In order to determine the A-Ci response curves the chamber light (1500µmol m^{-2}, saturation point for both species), and leaf temperature (28°C) conditions were kept constant. The chamber CO_2 concentrations were varied from 100 µmol mol^{-1} to 2000 µmol mol^{-1} and the relative assimilation rates and intercellular CO_2 concentrations measured when the total coefficient of variation (sum of the coefficient of variation of ΔCO_2; ΔH_2O and Δflow) was less than 1%. For the Light Response Curves the chamber CO_2 level (384 µmol mol^{-1}); leaf temperature (27°C) and humidity (15 mmol mol^{-1}) were kept constant and light intensity was varied from 2000 µmol m^{-2} s^{-1} to 0 µmol m^{-2} s^{-1} and the rate of assimilation was measured when the total coefficient of variation was less than 1%. The A-Ci and Light Response Curves were measured on leaves of similar age (mature leaf within the second whorl) of 5 plants for each species within each treatment, before treatment, after 30 days, 90 days and after 140 days of treatment. Data collected for the individual plants at any set interval were fitted with a line [equation: $y = a(1-\exp(b-c*x))$] using regression analysis and the generated constants were accepted if the R^2 value was > than 0.9. The mean of the constants a, b and c for the individual species within the treatments were used for comparison between treatments using an analysis of variance and multiple range test (Sheffe, $p<0.05$).

5.3.4 Leaf nutrients

Leaf samples were sent to the KwaZulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: Total leaf content of Ca; Mg; K; Na; P; Zn; Cu; Mn. The procedures used were based on that described by Hunter (1974). Leaf samples were dry ashed at 450°C overnight. The samples were then cooled and wet with few drops of distilled water, and 2ml of concentrated HCl was added to each 1g sample. Samples were dried on a water bath and then 25ml of 1M HCL solution was added using a Fortuna Optifix dispenser and then filtered through a Whatman No. 41, 9cm filter paper.

The reagent used for Ca; Mg; K and Na determination was a strontium solution consisting of 76g $SrCl_2.6H_2O$ in 10l of de- ionized water. Using a diluter a 1ml aliquot of the filtrate was added to 24ml of the strontium solution, this was then used to determine element content with the following instrument settings of a Varian Spectra A 220FS atomic absorption spectrophotometer. Ca was determined at 422.7nm, current of 4mA and a slit width of 0.5nm; Mg at a wavelength of 285.2nm, current of 4mA and slit width of 0.5nm; K at a wavelength of 766.5nm, current of 5mA and slit width of 1nm; and Na at a wavelength of 589.0nm, current of 10mA and slit width of 0.5nm. For the determination of Zn, Cu and Mn the undiluted filtrate in 1M HCL was used with the following instrument settings: Zn, Cu and Mn were all determined at a current of 5mA and a slit width of 1nm with wavelengths of 213.9nm; 324.8nm and 279.5nm used respectively.

In order to determine leaf phosphate concentration a 2ml aliquot of the filtrate strontium solution was added to 8ml de-ionized water and 10ml of P colour reagent. This was allowed to stand for 30 minutes and read on a spectrophotometer at 680nm. The P colour

reagent was prepared by making a solution with 15g ammonium molybdate ($(NH_4)_6Mo_7O_{24}4H_2O$)) in 600ml distilled water. This was added to an acid antimony solution, made up of 2g antimony potassium tartrate ($C_4H_4KO_7Sb$. ½H2O) with 800ml distilled water and 300ml concentrated H_2SO_4, and made up to a volume of 2*l* with distilled water. From this stock solution the P colour reagent was made by diluting 150ml of the stock solution with 1l of a solution containing 1g gelatin and 1g ascorbic acid.

5.3.5 Root morphology

After the experimental treatment period a 10cm wide vertical section of the fumigation chamber wall, of each chamber, was removed for observing rooting pattern. The exposed profile was covered with clear plastic and the pattern of the exposed roots traced. The traced profiles were then used to determine the maximum root branching order for each tree so as to determine if any differences in rooting response was caused by the different soil gas treatments. The branching habit of roots has very important implications for the performance of a root system in unfavourable conditions. The development of lateral roots starts within the protective cylinder of the main root endodermis, thus a reduction in laterals can be indicative of stress within the root cortex (Scott Russell, 1977)

The vertical sections of the chambers were replaced and held in position with wide adhesive tape. Six of the 10 replicates / species / treatment were divided into 5cm horizontal intervals (Total 7 segments per pot). Each 5cm section was successively removed from the chamber top using an angle grinder, which allowed the slicing of the pot wall, soil and roots to be completed accurately and efficiently. The soil from each section was washed through a 2mm sieve so as to separate the roots. For the upper two 5cm

intervals which intersected with the original net bagged root ball, only the soil and roots on the outside of the bag were removed at this time. This ensured that the new root growth under experimental treatment conditions was collected separately. The roots collected from each of the 7 sections and from within the net bag were oven dried at 105°C and weighed separately. The root mass was expressed as a ratio of the soil volume within each profile section in order to compensate for the slightly conical shape of the pot. These data as well as the root mass per section were used to determine a root biomass depth profile, maximum rooting depth and total root biomass. In determining the root mass depth profile there was a concern that the slight conical shape of the fumigation chambers may bias the results, therefore the data was analysed using root mass and root mass expressed as a ratio of soil volume. No difference in the results between the two ways of measuring the root profile was found, the more meaningful root mass results are presented here. The root system for the other 4 replicates was kept intact and the soil was carefully washed from the roots. These roots which were then sampled for porosity and microscopy measurements. After porosity and microscopy measurements were completed the sampled root material was dried and weighed and added to the dry weight of the remaining root material for each of the 4 replicates, providing total root biomass data for each plant.

5.3.6 Porosity

Four replicate 5cm sections of stem sampled just above the fumigation chamber and fresh seminal root material were used for tissue porosity measurements. Porosity measurements were done using Archimedes' principle as described by Raskin (1983). The principle states that the buoyant force acting upon a body immersed in a fluid is equal to the weight of the fluid displaced by the body. Thus, by determining the mass of a body in air, then measuring the positive buoyancy mass of the body in water of known density (0.99707g

The rate of stem height and diameter increase over the 140 days declined in all of the treatments including the control. This decline was expected, as newly potted plants will start off with a rapid growth that will stabilise with time. The decline was also attributed to the approach of winter and the shorter day lengths and lower sunlight intensity. In terms of the rate of height increase and the total height increase after 140 days there were no significant ($p>0.05$) differences between the treatments for either of the species (Figure 5.14 and 5.15).

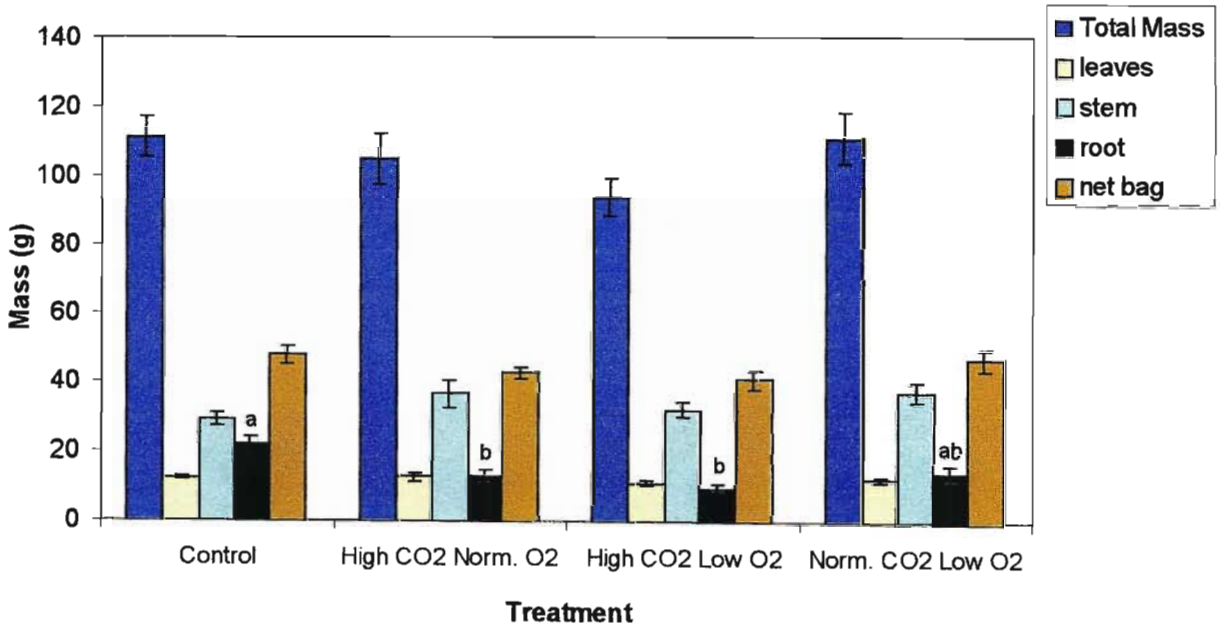


Figure 5.12: Tree total mass allocation for *Barringtonia* (n=10) after 140 days experimental treatment. Significant ($p<0.05$) differences between treatments for new root growth shown by a change in letter. There were no other significant differences between treatments.

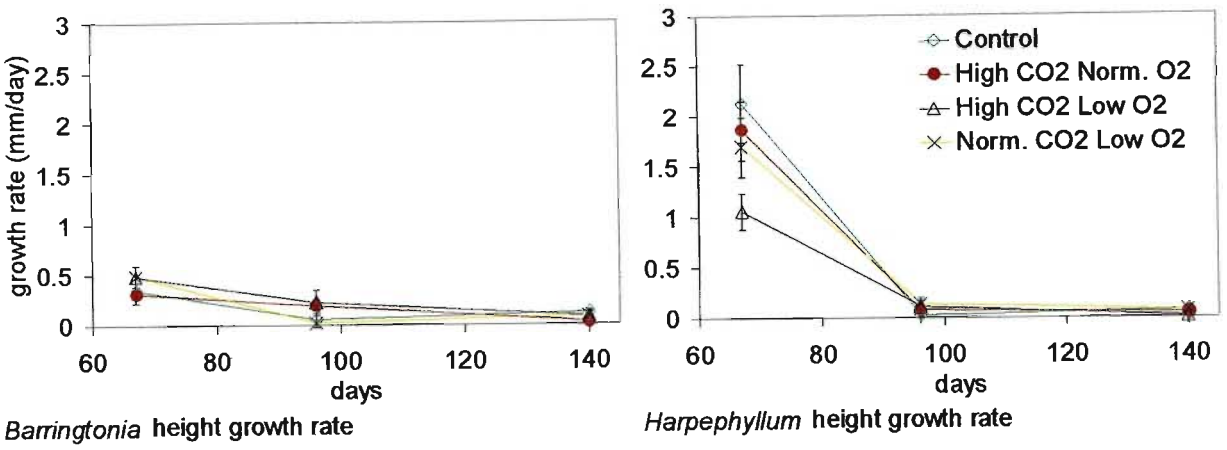


Figure 5.14: Rate of stem height extension for *Harpephyllum* and *Barringtonia*. No significant ($p>0.05$) differences between treatments at any point in time for either species.

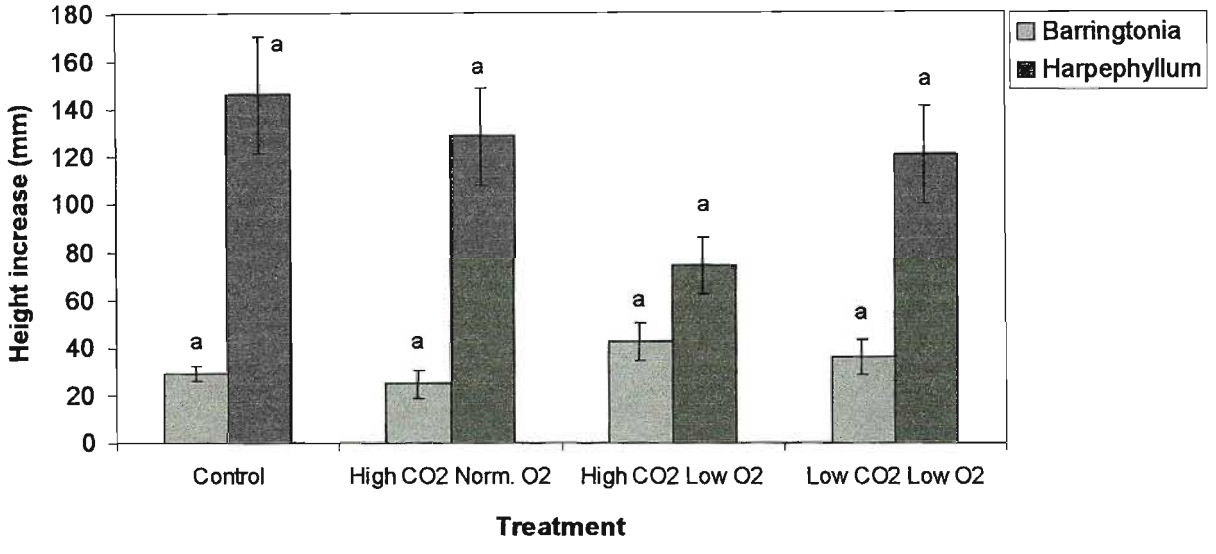


Figure 5.15: Total mean (±Std. Error) stem height increase after 140 days for *Barringtonia* and *Harpephyllum*. No significant ($p>0.05$) differences in height increase within species between treatments. Change in letter within species represents significant difference.

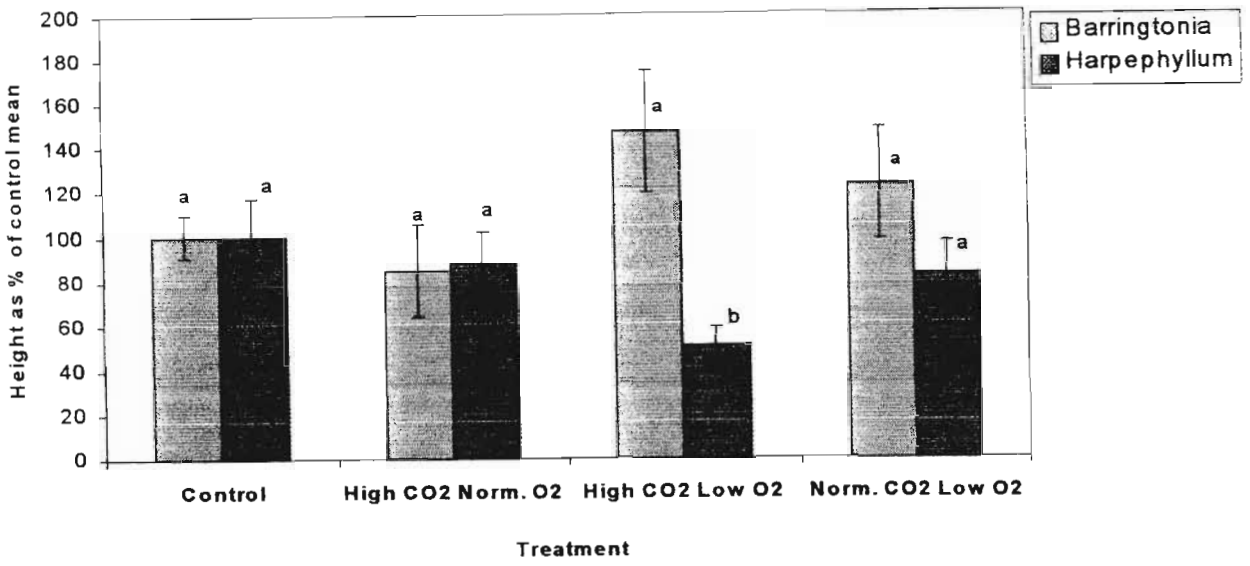


Figure 5.16: Total height increase expressed as a percentage of the control used to compare species within treatments. Significant ($p<0.05$) differences for each species within treatments shown by a change in letter.

In terms of the rate of stem diameter increase, *Barringtonia* in the normal CO_2 low O_2 treatment had an initial significantly ($p<0.05$) higher rate of increase measured at 70 days. This rate was not maintained and after 100 days the rate was not significantly ($p>0.05$) different from the control (Figure 5.17a). However, this initial high rate of stem diameter increase resulted in a significantly ($p<0.05$) higher total stem diameter increase relative to the control even after 140 days (Figure 5.17b). In the elevated CO_2 treatments the rate of stem diameter increase, although not initially as high as the normal CO_2 low O_2 treatment at 70 days, did not decline steeply and maintained a relatively high rate which became significantly ($p<0.05$) higher than the control when measured at 140 days. In fact the rate of stem diameter increase in the elevated CO_2 normal O_2 treatment was significantly ($p<0.05$) higher than the normal CO_2 low O_2 treatment. However, due to the initial low rate of increase in the elevated CO_2 treatments the actual total stem diameter increase over the 140 days was higher but not yet significantly ($p>0.05$) different from the control. The results suggested elevated CO_2 caused a slower, more sustained increase in the rate of stem diameter increase, even in the presence of low O_2, whilst low O_2 and normal CO_2 caused

an initial very high but non-sustained rate of stem diameter increase. Given a longer experimental period the rates of diameter increase suggest that the diameters of both the elevated CO_2 treatments would become greater than the normal CO_2 low O_2 treatment.

In the normal CO_2 low O_2 and the elevated CO_2 normal O_2 treatments *Harpephyllum* had an initial significantly ($p<0.05$) high rate of stem diameter increase relative to the control but this was not maintained (Figure 5.18a). However, it did result in a significantly ($p<0.05$) higher total stem diameter increase in these treatments relative to the control (Figure 5.18b). This suggested that the increase in *Harpephyllum* stem mass discussed earlier was primarily due to diameter increase. In the elevated CO_2 low O_2 treatment there was no initial high rate of stem diameter increase relative to the control (Figure 5.18a). This resulted in no significant ($p>0.05$) difference in total stem diameter increase relative to the control and explained the lack of increase in stem mass. Unlike the other treatments which showed an increase in stem mass equivalent to the reduced root mass, which resulted in no difference in total mass relative to the control, the elevated CO_2 low O_2 resulted in no enhancement of stem mass resulting in a significantly lower total mass (Figure 5.13). The results show that *Harpephyllum* in the high CO_2 low O_2 treatment had no initially high stem diameter increase rate or higher stem mass and had an apparent lower stem height increase, whilst the other treatments showed a definite stem growth response. This suggested a possible synergistic effect resulting from the combination of elevated CO_2 with low O_2 causing no enhanced stem growth that was a response measured in the other treatments. In comparing the stem diameter of the two species *Harpephyllum* had an overall greater stem diameter, however, in terms of relative stem diameter increase *Barringtonia* had a significantly ($p<0.05$) higher increase in the elevated CO_2 low O_2 and the normal CO_2 low O_2 treatments. Both species showed a similar stem growth response to

treatment conditions, however *Barringtonia's* response was slower and more sustained and was not inhibited by the combination of elevated CO_2 and low O_2.

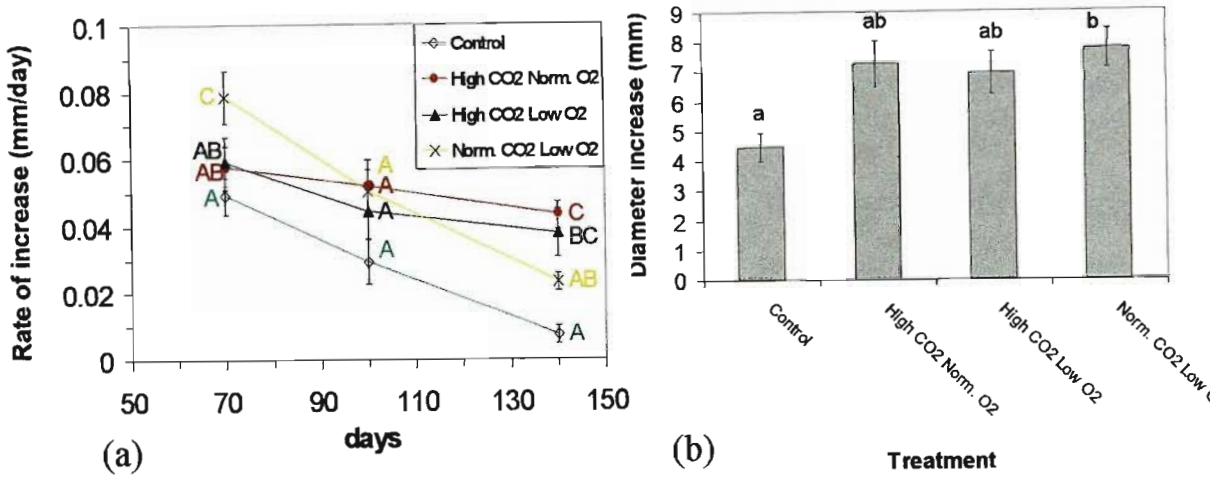


Figure 5.17: (a) Rate of *Barringtonia* stem diameter increase during experiment. Significant ($p<0.05$) differences between treatments at any individual point in time shown by a change in letter. (b) Total stem diameter increase after 140 days (i.e. $T_{140} - T_0$) in *Barringtonia*, significant ($p<0.05$) differences between treatments shown by a change in letter.

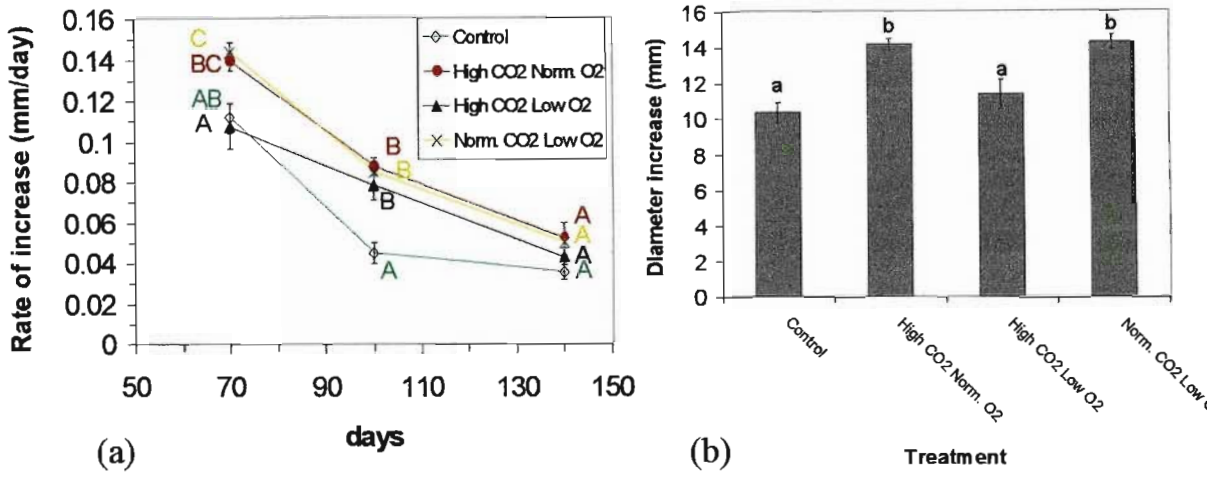


Figure 5.18: (a) Rate of *Harpephyllum* stem diameter increase during experiment. Significant ($p<0.05$) differences between treatments at any individual point in time shown by a change in letter. (b) Total stem diameter increase after 140 days (i.e. $T_{140} - T_0$) in *Harpephyllum*, significant ($p<0.05$) differences between treatments shown by a change in letter.

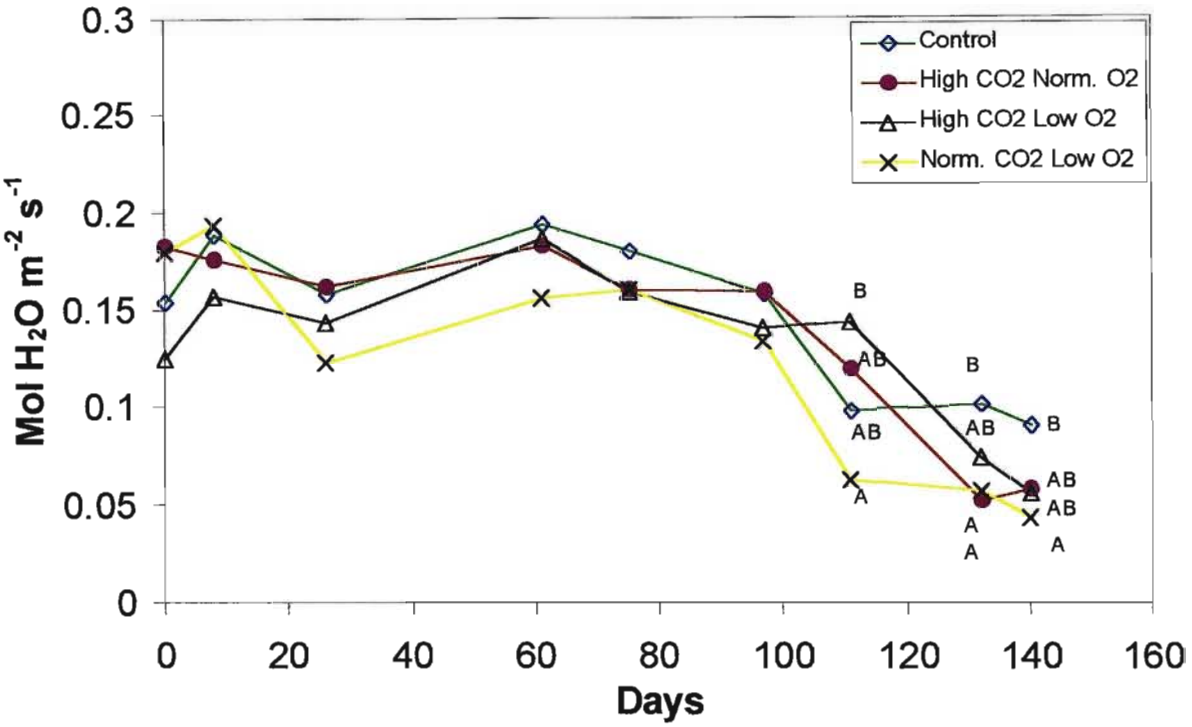


Figure 5.19: *Barringtonia* stomatal conductance, no significant ($p>0.05$) differences between treatments up to 111 days, significant ($p<0.05$) differences between treatments shown by a change in letters.

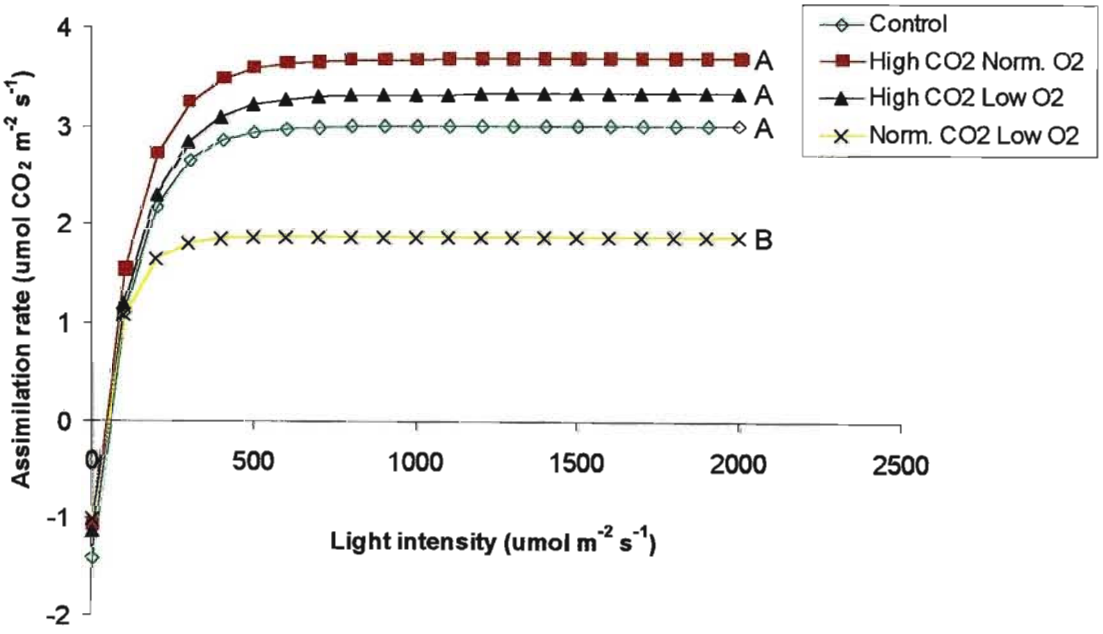


Figure 5.20: *Barringtonia* light response curves (140 day) for the different treatments. Significant ($p<0.1$) differences in regression constant "a" shown by a change in letter. There were no significant ($p>0.1$) differences in the regression constants "b" and "c".

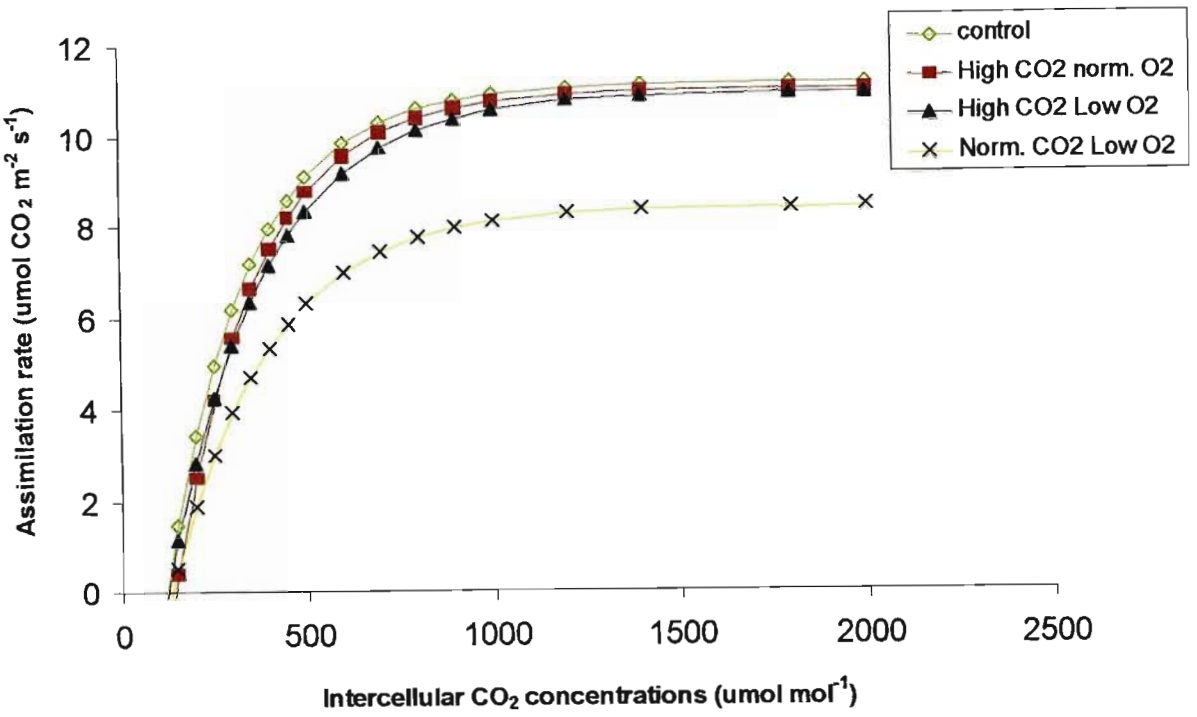


Figure 5.21: *Barringtonia* Aci response curves (140 day) for the different treatments. No significant ($p>0.1$) differences in regression constants.

Unlike *Barringtonia*, the stomatal conductance of *Harpephyllum* in the normal CO_2 low O_2 treatment showed an initial decline but the difference relative to the control became non-significant ($p>0.05$) after 111 days of fumigation (Figure 5.22). Also unlike *Barringtonia* the light response and Aci curves for the normal CO_2 low O_2 treatment were very similar to the control, with no significant ($p>0.1$) difference in the regression constant values at any point in time. However, after 75 days of fumigation the elevated CO_2 treatments showed significantly ($p<0.05$) lower stomatal conductance values relative to the control (Figure 5.22). There was only one exception at 111 days when there was no significant difference ($p>0.05$) in stomatal conductance between any of the treatments. This was attributed to relatively high data variability, due to patchy cloud cover, and was not considered an important break in the general trend. In fact stomatal conductance in the elevated CO_2 treatments dropped to 0.02 mol H_2O m^{-2} s^{-1} at 140 days and prevented the accurate

calculation of intercellular CO_2 concentrations and Aci response curves could no longer be generated, thus data for Aci response curves are not given. However up to this point there were no significant differences ($p>0.1$) in Aci regression constants between the treatments. The light response curves also showed no significant ($p>0.1$) differences, except for the 140 day measurements which showed a significantly ($p<0.1$) lower regression constant "a" in the elevated CO_2 treatments relative to the control (Figure 5.23). Interestingly the "a" constant in the low O_2 treatment without elevated CO_2 did not differ significantly ($p>0.1$) from the control. The results suggested that *Harpephyllum* stomatal conductance and the light response were affected by elevated CO_2 and there was no response to low O_2, whilst *Barringtonia* appeared to be most affected by the low O_2 conditions and elevated CO_2 appeared to alleviate this effect. *Harpephyllum* had significantly ($p<0.05$) lower relative "a" constant in the elevated CO_2 treatments whilst in the normal CO_2 low O_2 treatment *Barringtonia* had a significantly ($p<0.05$) lower relative "a" constant in comparison to *Harpephyllum*. The relative stomatal conductance results, although not significant ($p>0.05$) also suggested that elevated CO_2 was having a greater impact on *Harpephyllum* than *Barringtonia*, whilst low O_2 in the absence of elevated CO_2 was having an effect on *Barringtonia*.

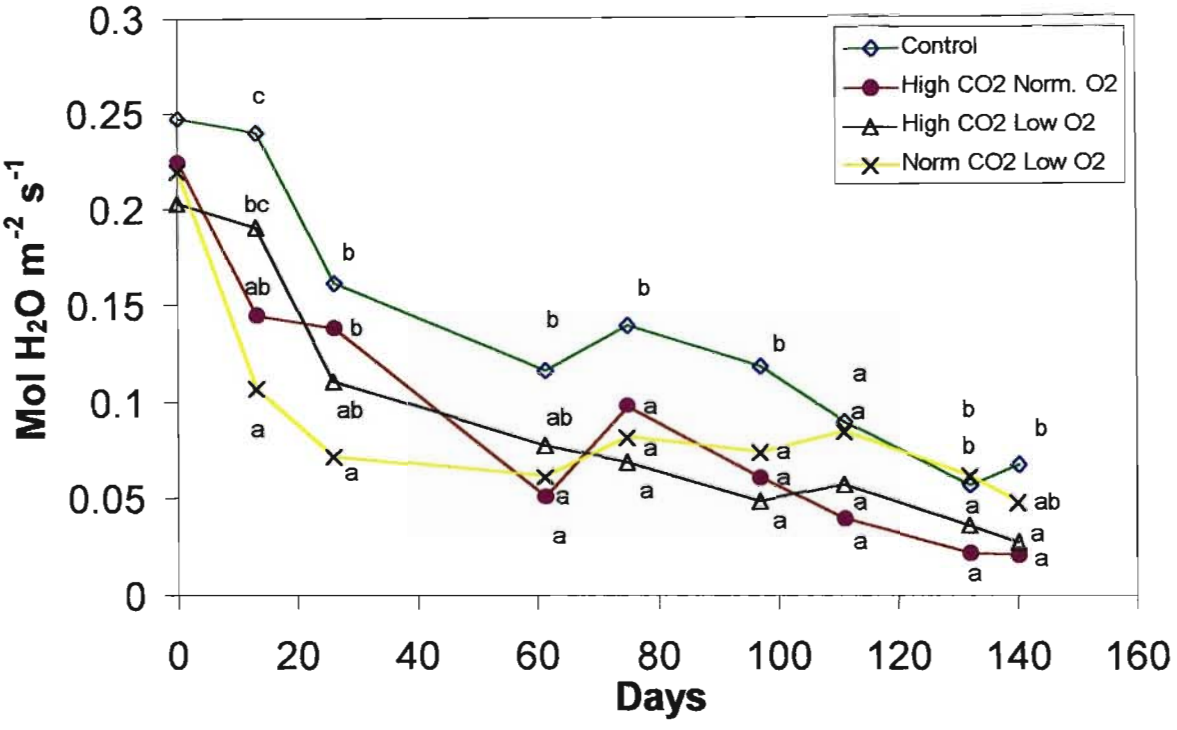


Figure 5.22: *Harpephyllum* stomatal conductance, significant ($p<0.05$) differences between treatments at any point in time shown by a change in letter.

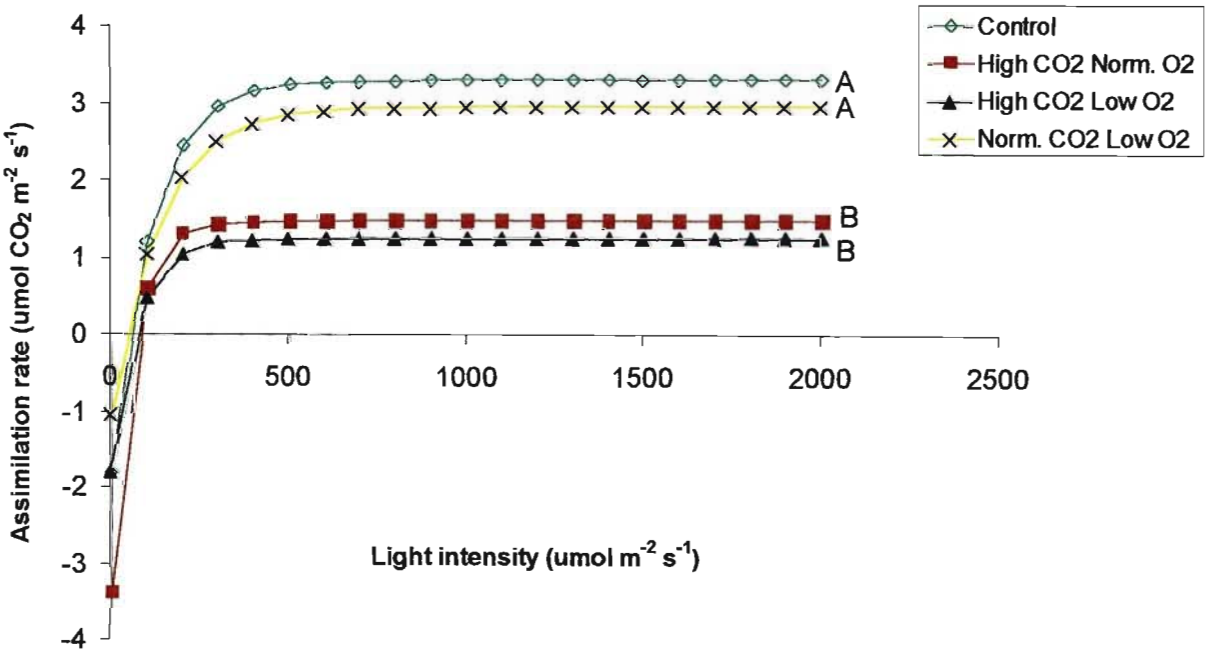


Figure 5.23: *Harpephyllum* light response curves (140 day) for the different treatments. Significant ($p>0.1$) differences in regression constant "a" shown by change in letter.

Table 5.2: Mean nutrient concentrations (mg/Kg ± standard error) in *Barringtonia* leaves after 140 days of the different gas regimes. Significant ($p<0.05$) differences between treatments for each element shown by a change in letter.

Nutrient	Control	High CO_2 Norm. O_2	High CO_2 Low O_2	Norm. CO_2 Low O_2
Ca	13300 ±900 a	13200 ±600 a	11100 ±400 a	12200 ±800 a
Mg	7200 ±200 b	6300 ±500 ab	5500 ±300 a	6700 ±500 ab
K	6700 ±400 a	10100 ±1100 a	10000 ±900 a	7500 ±800 a
Na	600 ±40 a	1000 ±200 ab	1400 ±200 b	1300 ±200 b
P	1700 ±200 a	1200 ±100 a	1200 ±100 a	1200 ±200 a
Zn	72.3 ±6.8 a	63.7 ±7.5 a	65.3 ±8.7 a	60.4 ±5.6 a
Cu	11.8 ±1.4 b	6.0 ±1.0 a	8.5 ±1.5 ab	6.6 ±1.1 ab
Mn	248.3 ±16.2 a	276.3 ±30.6 a	225.1 ±19.4 a	235.1 ±21 a

Table 5.3: Mean nutrient concentrations (mg/Kg ± standard error) in *Harpephyllum* leaves after 140 days of the different gas regimes. Significant ($p<0.05$) differences between treatments for each element shown by a change in letter.

Nutrient	Control	High CO_2 Norm. O_2	High CO_2 Low O_2	Norm. CO_2 Low O
Ca	27000 ±1200 a	22800 ±1200 a	21200 ±1300 a	23400 ±1900 a
Mg	2000 ±100 ab	1600 ±200 a	1700 ±100 a	2400 ±200 b
K	9200 ±400 b	6600 ±500 a	7100 ±400 a	7500 ±400 ab
Na	400 ±80 a	300 ±40 a	500 ±40 a	500 ±50 a
P	900 ±60 a	500 ±30 b	900 ±60 a	800 ±60 a
Zn	37.2 ±4.1 a	24.9 ±4.4 a	40.6 ±6.8 a	27.0 ±4.2 a
Cu	6.0 ±0.3 ab	5.0 ±0.4 a	5.5 ±1.0 a	8.5 ±0.6 b
Mn	49.1 ±5.5 ab	31.3 ±1.9 a	46.7 ±4.4 ab	53.1 ±4.1 b

In terms of relative nutrient content compared between the species within the treatments there were few significant ($p<0.05$) differences. However, of these differences it was noted that the *Barringtonia* generally had higher relative leaf nutrient contents except in the case

of Cu and Mn (Table 5.4). In general the leaf nutrient results suggested that the effect of the treatments was less marked in *Barringtonia* as only 3 nutrients compared to 5 in *Harpephyllum* had any significant differences between the treatments and the overall impact of the gas conditions was relatively vague.

Table 5.4: Comparison between species of leaf element content expressed as a percentage of the mean of the control for each species.

Element	***Barringtonia***		***Harpephyllum***		
Ca	**Mean %**	**Std. Error**	**Mean %**	**Std. error**	***P value***
High CO_2 Norm. O_2	99.3	4.3	84.4	4.3	0.028 *
High CO_2 Low O_2	83.3	3.0	78.5	4.8	0.402
Norm. CO_2 Low O_2	91.8	5.9	86.6	6.9	0.582
Mg					
High CO_2 Norm. O_2	87.7	6.6	80.7	11.6	0.596
High CO_2 Low O_2	76.4	4.4	84.7	4.6	0.204
Norm. CO_2 Low O_2	93.7	6.9	116.7	9.3	0.67
K					
High CO_2 Norm. O_2	150.9	17.0	72.3	5.7	0.001 *
High CO_2 Low O_2	148.9	13.3	77.5	4.8	0 *
Norm. CO_2 Low O_2	112.3	12.2	81.6	4.9	0.026 *
Na					
High CO_2 Norm. O_2	165.7	28.8	84.4	10.5	0.023 *
High CO_2 Low O_2	240.7	31.9	112.5	9.3	0.001 *
Norm. CO_2 Low O_2	220.3	28.4	115.0	11.3	0.002 *
P					
High CO_2 Norm. O_2	70.8	7.0	58.9	3.4	0.163
High CO_2 Low O_2	68.5	4.6	108.0	6.9	0 *
Norm. CO_2 Low O_2	68.8	9.3	87.4	6.7	0.118
Zn					
High CO_2 Norm. O_2	88.1	10.4	66.9	11.8	0.196
High CO_2 Low O_2	90.3	12.0	109.1	18.3	0.401
Norm. CO_2 Low O_2	83.6	7.8	72.6	11.3	0.443
Cu					
High CO_2 Norm. O_2	50.8	8.8	83.3	6.3	0.01 *
High CO_2 Low O_2	72.0	12.7	91.7	17.3	0.372
Norm. CO_2 Low O_2	55.6	9.3	141.7	10.0	0 *
Mn					
High CO_2 Norm. O_2	111.3	12.3	63.6	3.9	0.003 *
High CO_2 Low O_2	90.7	7.8	95.1	8.9	0.71
Norm. CO_2 Low O_2	94.7	8.4	108.1	8.3	0.274

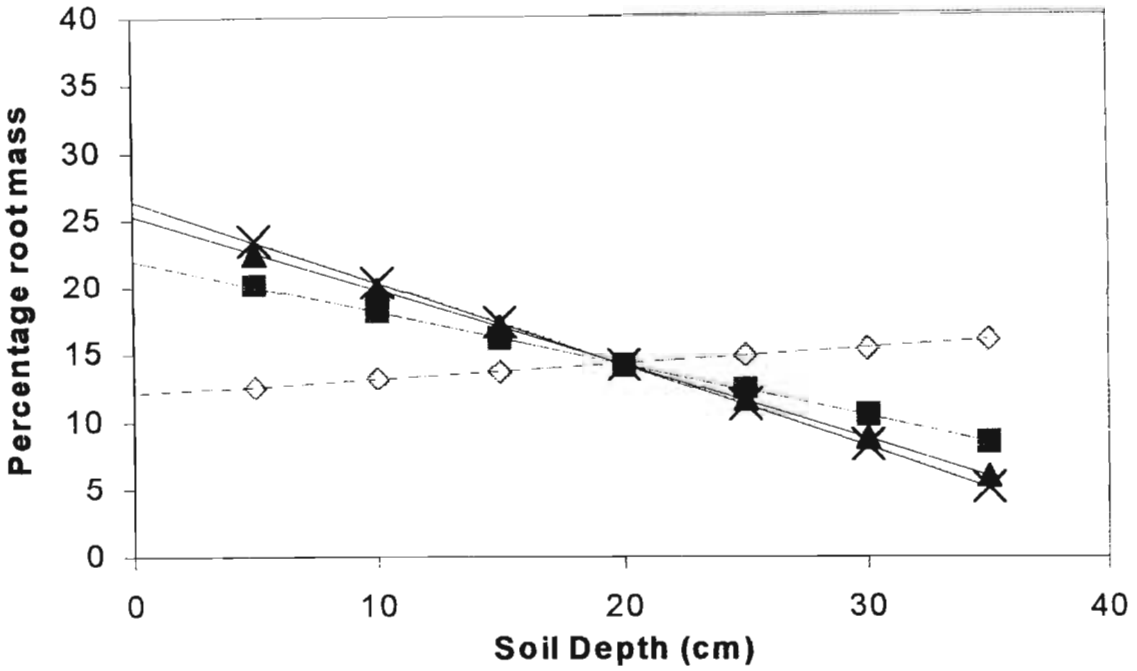
* significant difference between species (ANOVA $p<0.05$)

5.4.3 Root morphology

The mass of new roots within each of the 7 depth intervals was expressed as a percentage of the total new root mass. Using linear regression, the y intercepts and gradients for the root mass depth profiles of 6 trees / species / treatment were determined. The mean gradient and mean y intercept for each species for each treatment was calculated. A comparison of the mean gradients and mean y intercepts between treatments, within species, was made using analysis of variance. The regression lines generated from the mean gradient and y intercept for each treatment are shown for *Barringtonia* in Figure 5.24 and *Harpephyllum* in Figure 5.25. The depth profile for *Barringtonia* roots showed a significant ($p<0.05$) reverse in gradient relative to the control for the low oxygen treatments. Whilst *Harpephyllum* showed a significant ($p<0.05$) reverse in gradient for all the treatments relative to the control, indicating that the proportion of root mass reduced with depth instead of increasing.

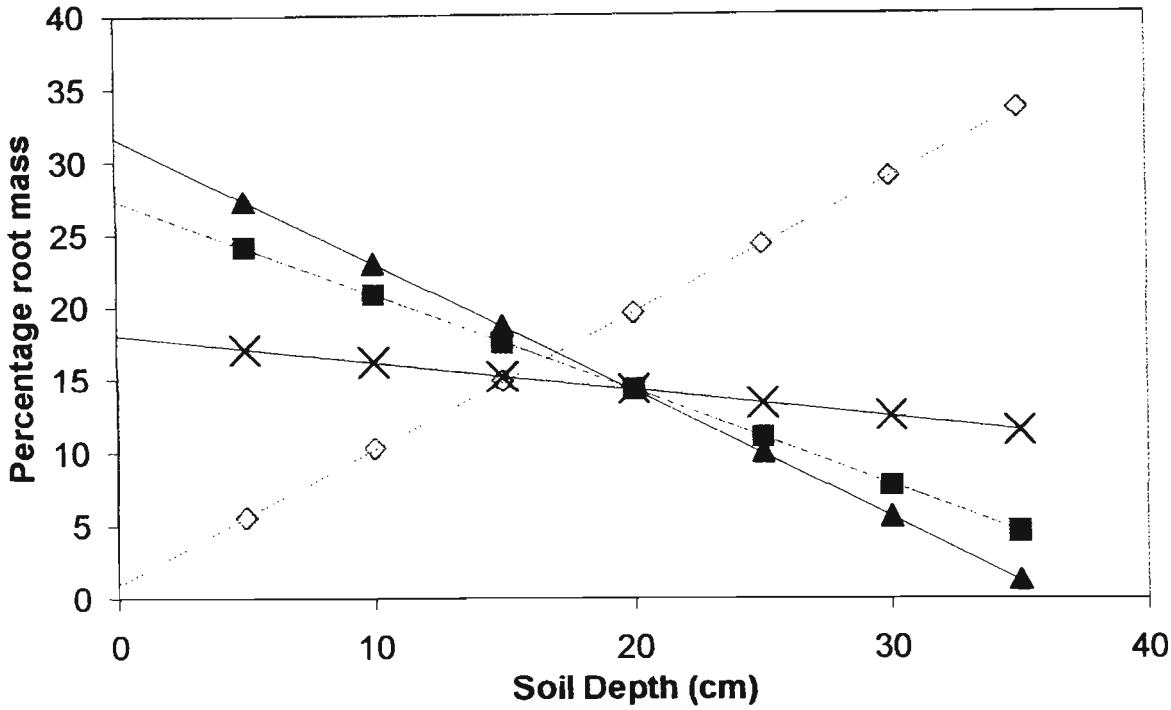
The y intercept values for *Barringtonia* indicated that the low oxygen treatments had significantly ($p<0.05$) higher proportions of root mass near the soil surface relative to the control, whilst the high CO_2 normal O_2 treatment was higher but not significantly ($p>0.05$) different from the control (Figure 5.24). In *Harpephyllum* the y intercept values indicated a significantly higher proportion of root mass near the surface for all the treatments relative to the control (Figure 5.25). The elevated CO_2 treatments had a greater proportion of roots mass near the surface relative to the normal CO_2 low O_2 treatment. However, only the elevated CO_2 low O_2 was significantly ($p<0.05$) different (Figure 5.25).

In summary, low oxygen conditions resulted in an overall greater proportion of roots near the soil surface for both species. However, *Harpephyllum*, unlike *Barringtonia*, had an even greater shift in the proportion of roots near the soil surface under elevated CO_2 conditions, even in the presence of normal oxygen levels. Although, the treatments had a significant effect on the root biomass depth profile of both species there was no apparent effect on the root branching habit. There were no significant ($p>0.05$) differences found between the mean ($n=10$) maximum root branching orders measured between the treatment or the species (Figure 5.26). The roots seen within the traced profiles showed an average of three levels of branching, however, it was not possible to establish if those roots seen were already of a higher branch order before they became visible in the exposed profile. Therefore the technique possibly lacked the ability to detect subtle changes in root branching that may have been caused by the treatments.



Treatment	Gradient	Y intercept
◇ Control	A	A
■ High CO2 Norm. O2	AB	AB
▲ High CO2 Low O2	B	B
× Norm. CO2 Low O2	B	B

Figure 5.24: *Barringtonia* root mass depth profile shown by regression lines generated from the mean gradient and y intercept for each treatment. Legend shows significant differences between treatments in gradient or y intercept of regression lines by a change in letters (Sheffe multiple range test).



Treatment	Gradient	Y intercept
◇ Control	A	A
■ High CO2 Norm. O2	B	BC
▲ High CO2 Low O2	B	C
× Norm. CO2 Low O2	B	B

Figure 5.25: *Harpephyllum* root mass depth profile shown by regression lines generated from the mean gradient and y intercept for each treatment. Legend shows significant differences between treatments in gradient or y intercept of regression lines by a change in letters (Sheffe multiple range test).

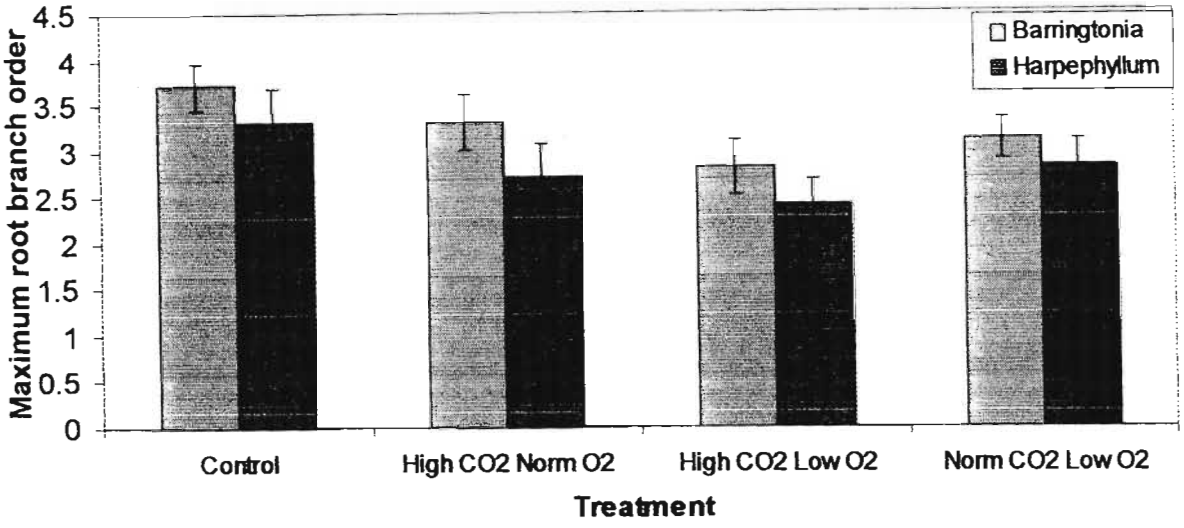


Figure 5.26: Mean (n=10) maximum root branch order for each treatment for both species. Error bar shows standard error of mean. No significant ($p>0.05$) differences between species or treatments.

5.4.4 Root and stem structure

The mean porosity of *Barringtonia* roots (range 8.9-13%) and stem (range 9.2-11.7%) was considerably, and significantly ($p<0.05$), higher across all treatments compared to *Harpephyllum* roots (range 0.1-2.3%) and stems (range 1.1-1.9%). There was no significant ($p>0.05$) difference between stem and root tissue porosity for *Barringtonia*, suggesting a possible high level of continuous interconnected intercellular air spaces in this species (Figure 5.27). The tissue porosity also appeared to be an inherent characteristic, as there was no significant ($p>0.05$) difference between any of the treatments (Figure 5.27). For *Harpephyllum* there was also no significant ($p>0.05$) difference in tissue porosity between the experimental treatments however, unlike the other treatments, in the control the roots had very low porosity and were significantly ($p<0.001$) lower than the stem (Figure 5.28).

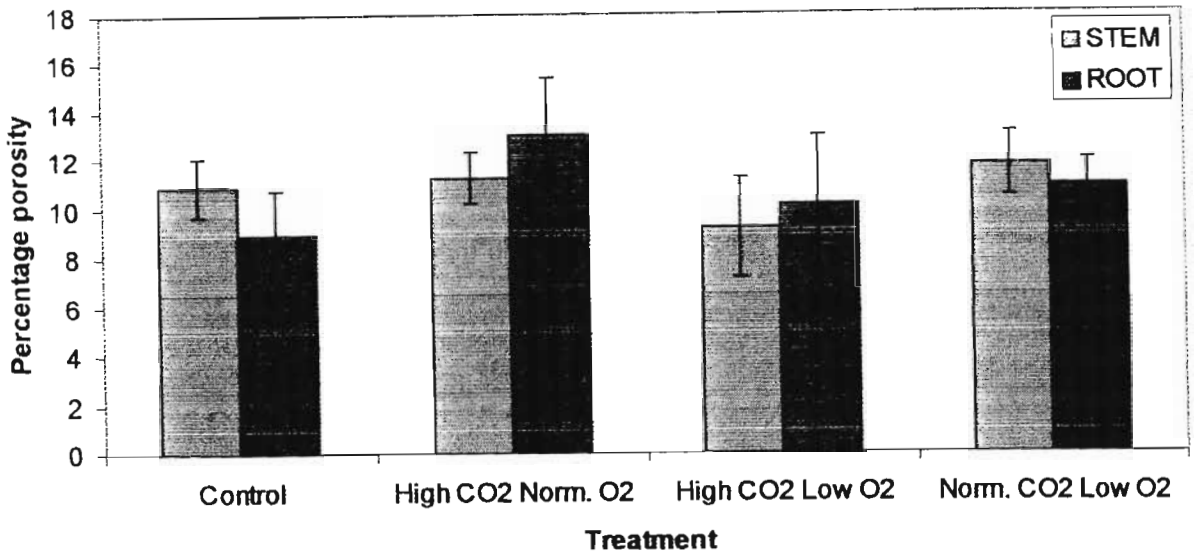


Figure 5.27: Mean with standard error of stem and root porosity for *Barringtonia*. No significant ($p>0.05$) differences between treatments or between stem and root within treatments.

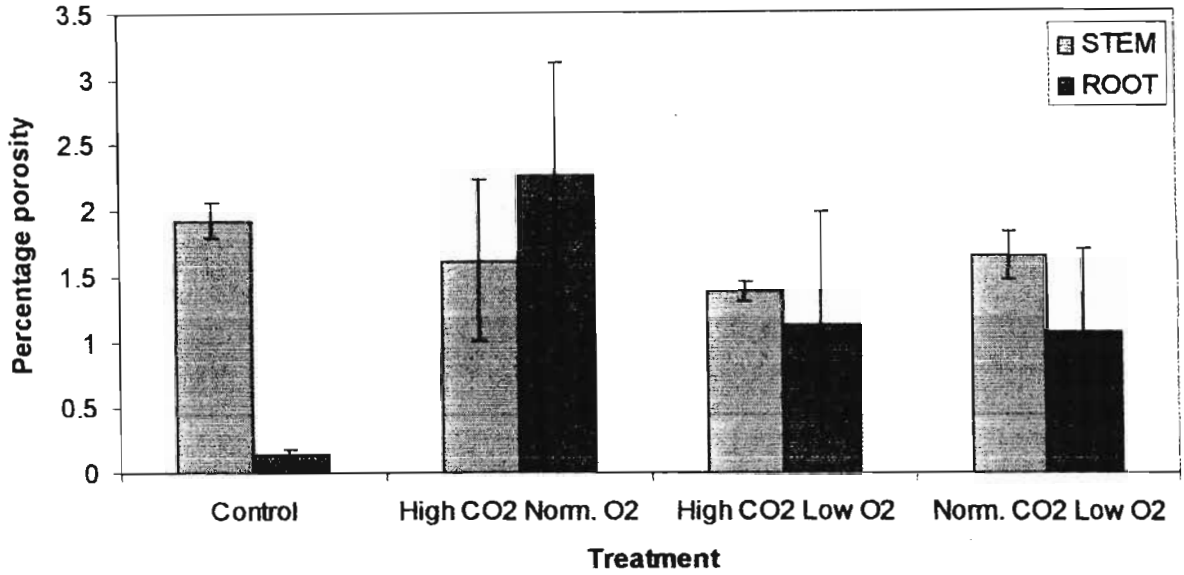


Figure 5.28: Mean with standard error of stem and root porosity for *Harpephyllum*. No significant ($p>0.05$) differences between the treatments or between root and stem within treatments, except for the control which had significant difference between stem and root ($p<0.001$).

These data for *Harpephyllum* suggests a possible increase in root porosity in the presence of gas treatments, however, the relatively large standard error of the mean root porosity

data, which explains the lack of significant difference between the control and the treatments, makes the data difficult to interpret. The results could suggest that the increase in root porosity was highly variable within the seminal roots indicating root cell die back rather than the formation of continuous interconnected intercellular air spaces.

The microscopical study of seminal root and stem material showed a marked difference in tissue structure between the two species. The key differences in the root tissue were the degree of secondary thickening and configuration of cortical cells. In *Barringtonia* the cortical cells were loosely packed in well ordered radial rows with each cell having four near neighbours to give a cubic cell packing arrangement and the appearance of successive concentric rings of cells within the cortex (Figure 5.29). The resultant intercellular spaces were shaped like a concave quadrangulus. The absence of single or several adjacent radial rows of cortical cells was apparent in all of the *Barringtonia* root tissue sampled, providing evidence of inherent lysigenous aerenchyma formation. No secondary thickening was apparent in any of the root sections observed for *Barringtonia* (Figure 5.29). There was no evidence of root anatomical differences between the treatments (Figure 5.31).

The *Harpephyllum* root tissue showed evidence of extensive secondary thickening and the formation of secondary xylem and phloem. The cortex and epidermis had been replaced by cork cambium that together with its derivatives, the phellogen and phellum, comprised the periderm. The cell structure of the root tissue appeared denser than that of *Barringtonia*, with very little intercellular air space and no evidence of aerenchyma formation (Figure 5.30). There was also no evidence of root anatomical differences between the treatments (Figure 5.32). There was no microscopical evidence to explain the high variability in root porosity in the root tissue exposed to the treatments.

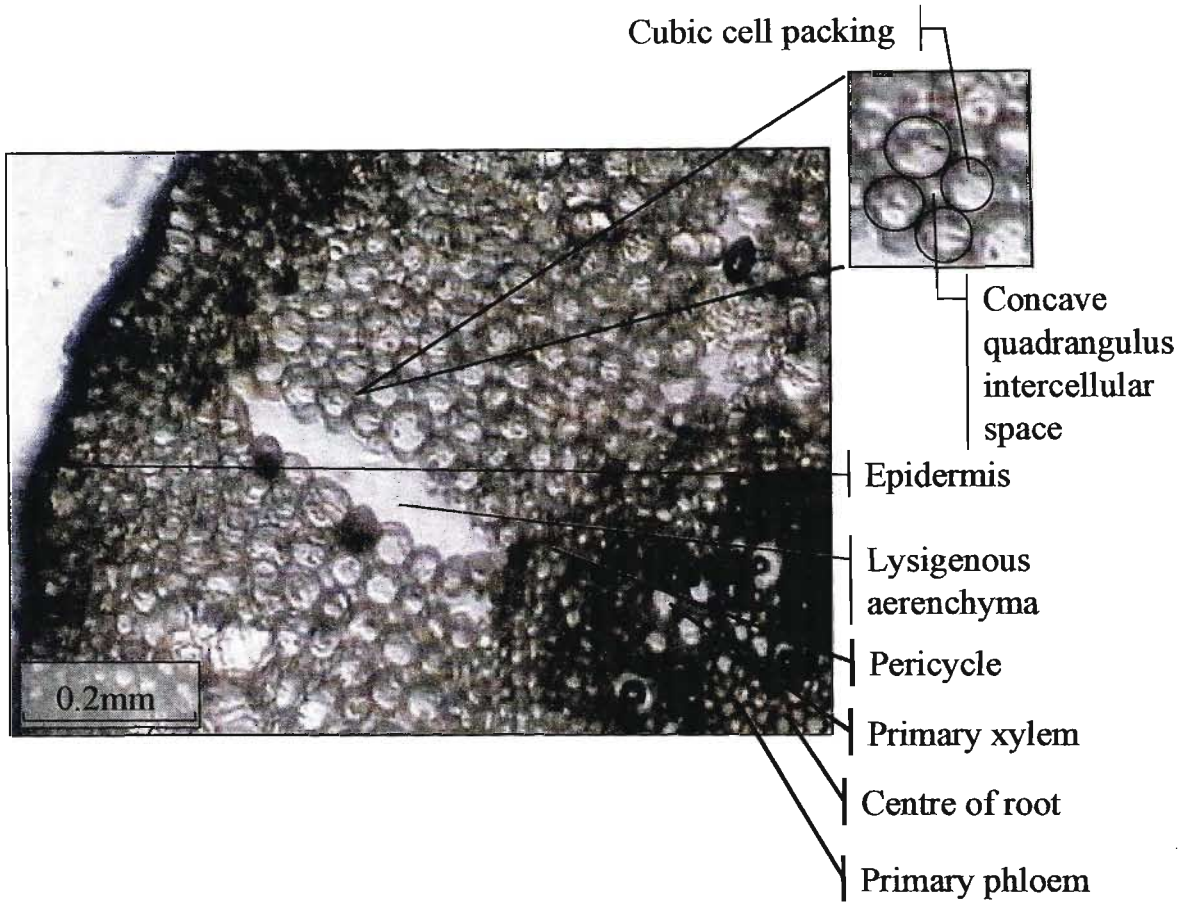


Figure 5.29: Cross section of *Barringtonia* seminal root showing cortical cell packing and the presence of aerenchyma

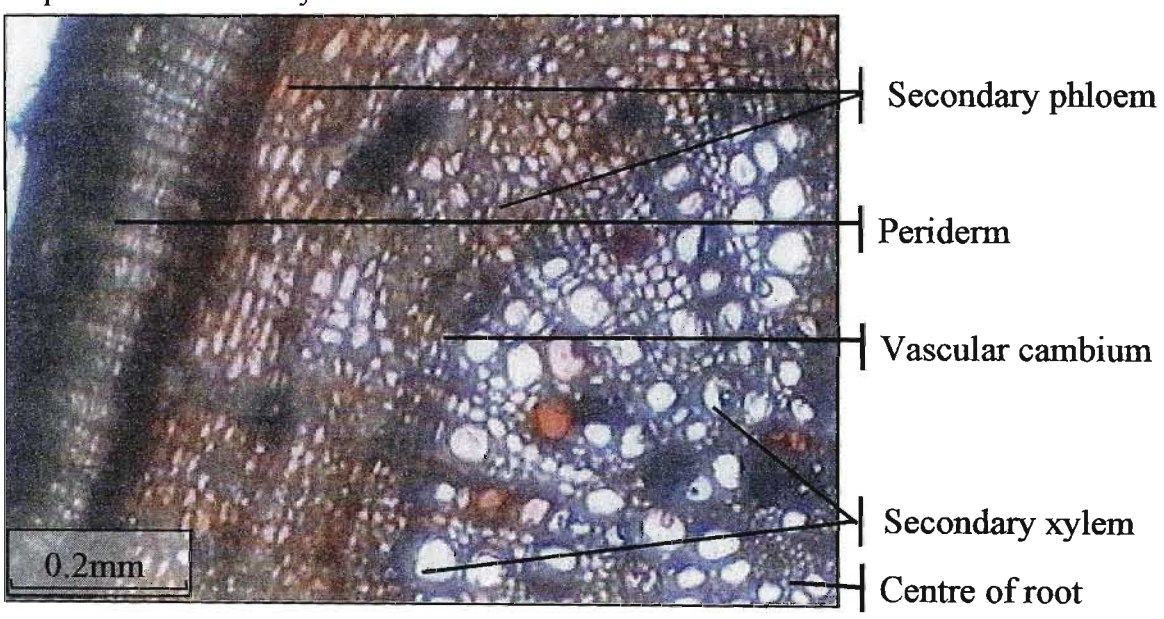
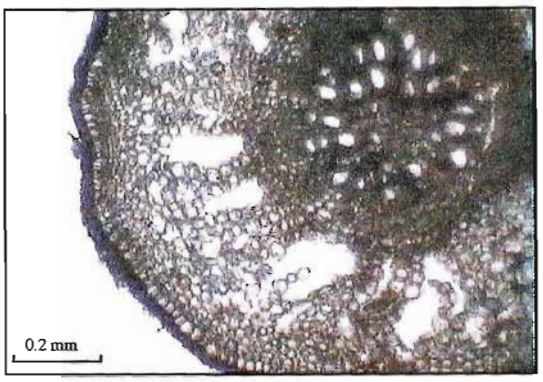
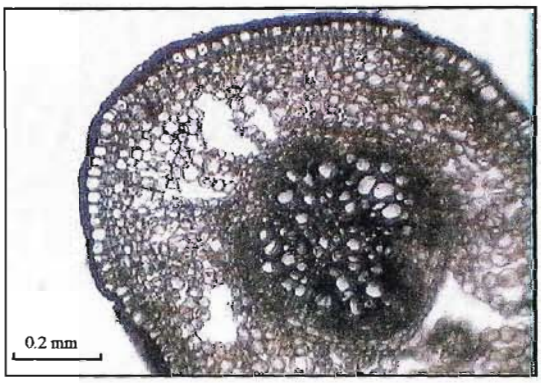


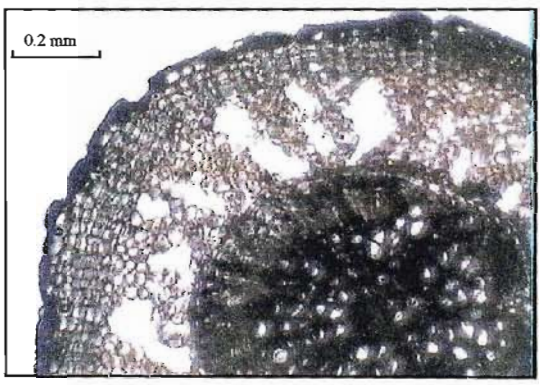
Figure 5.30: Cross section of *Harpephyllum* seminal root showing extensive secondary thickening and total loss of cortical cells and epidermis.



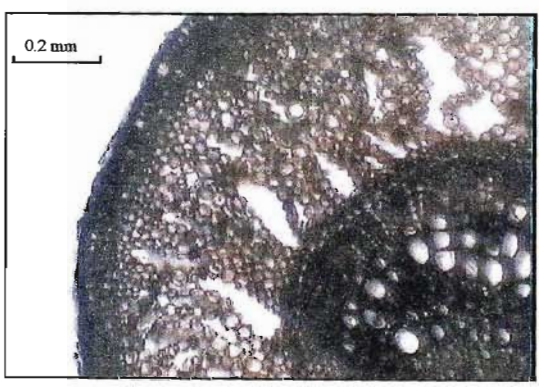
Control:
Barringtonia seminal
root cross section



High CO_2 Normal O_2:
Barringtonia seminal root cross section

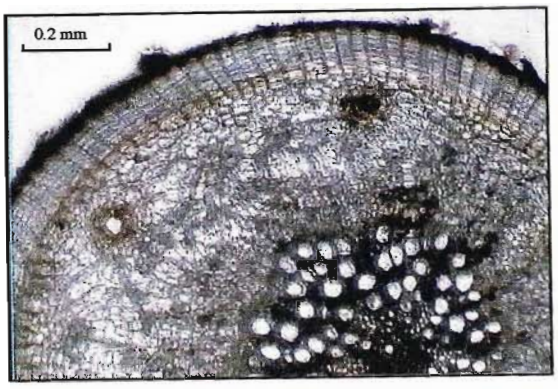


High CO_2 Low O_2:
Barringtonia seminal root cross section

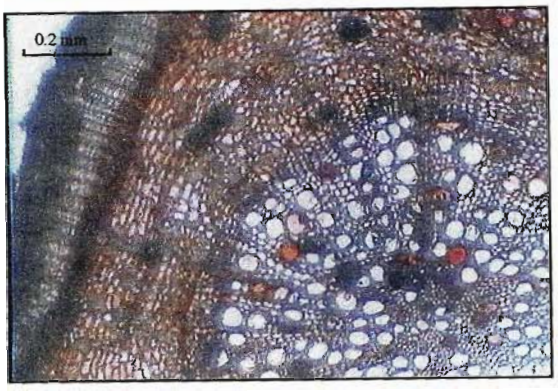


Normal CO_2 Low O_2:
Barringtonia seminal root cross section

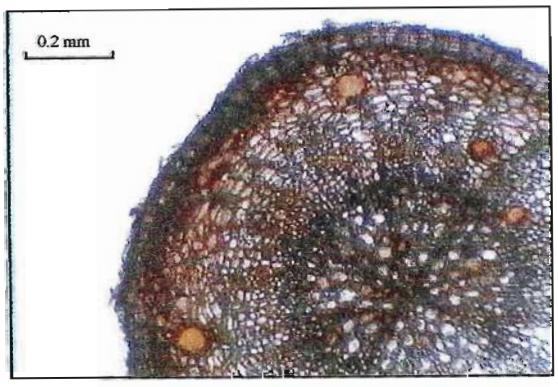
Figure 5.31: Light microscopy cross sections of representative examples of *Barringtonia* seminal roots from the different treatments, showing no treatment effect on root anatomy



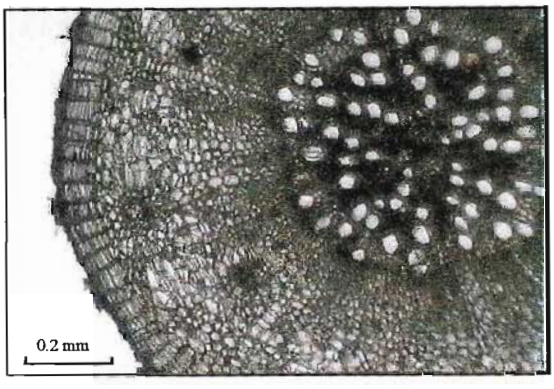
Control:
Harpephyllum seminal
root cross section



High CO_2 Normal O_2:
Harpephyllum seminal root cross section



High CO_2 Low O_2:
Harpephyllum seminal root cross section



Normal CO_2 Low O_2:
Harpephyllum seminal root cross section

Figure 5.32: Light microscopy cross sections of representative examples of *Harpephyllum* seminal roots from the different treatments, showing no treatment effect on root anatomy

In terms of the wood anatomy of the two species, there was a distinct difference in the level of wood fibres and overall tissue density. The *Barringtonia* stems had distinctly larger fibre cells and a more open wood structure when compared with the stem cross sections of *Harpephyllum.* (Figure 5.33 and 5.34). However, for both species there was no evidence of any differences in wood anatomy due to the treatments as seen in Figure 5.35.

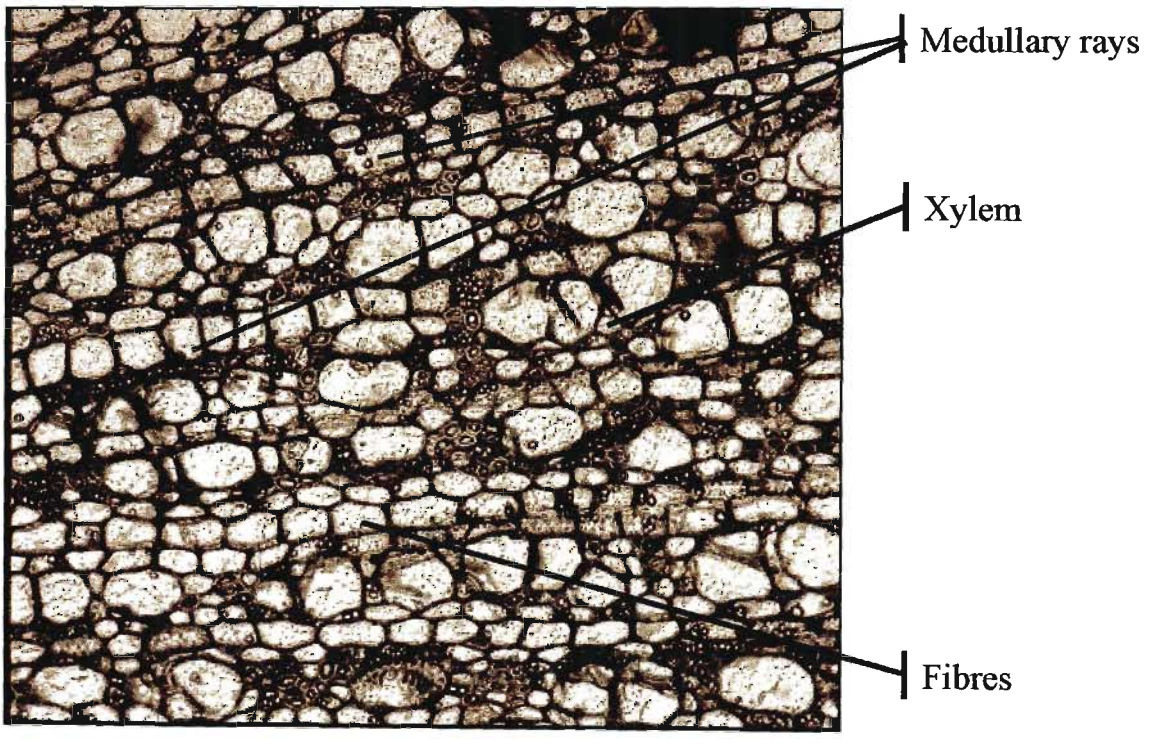


Figure 5.33: Cross section of *Barringtonia* stem, showing wood tissue structure (x25)

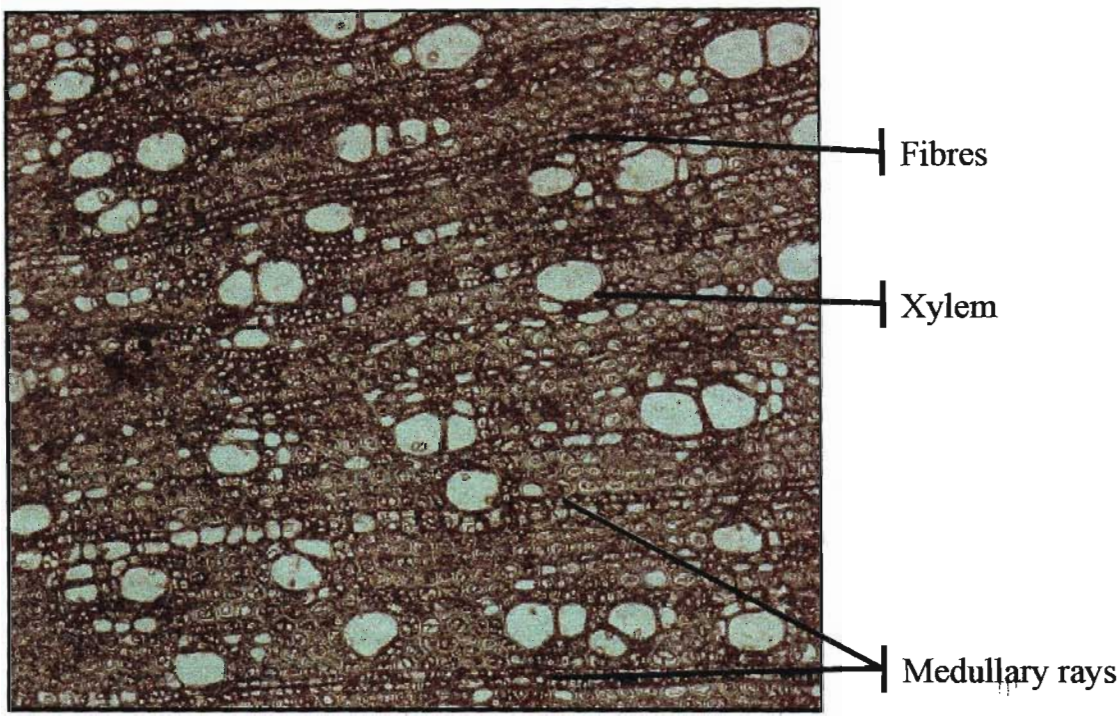
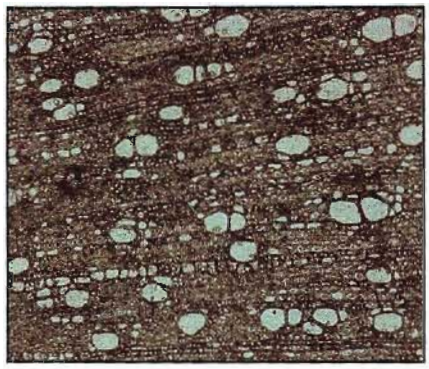
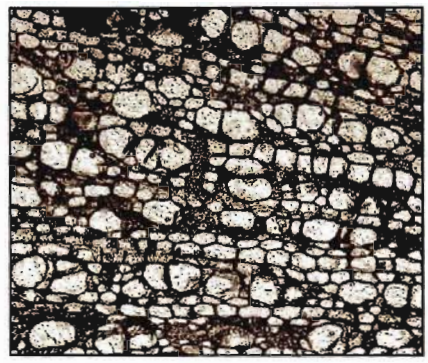


Figure 5.34: Cross section of *Harpephyllum* stem, showing wood tissue structure (x25)

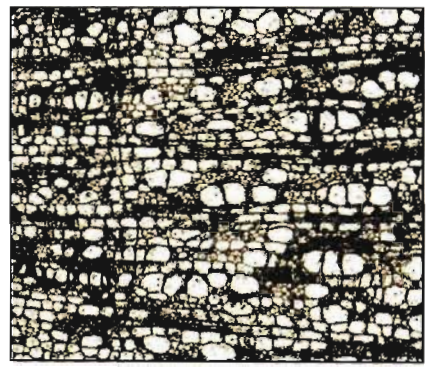


Control: *Harpephyllum*, stem cross section X 10



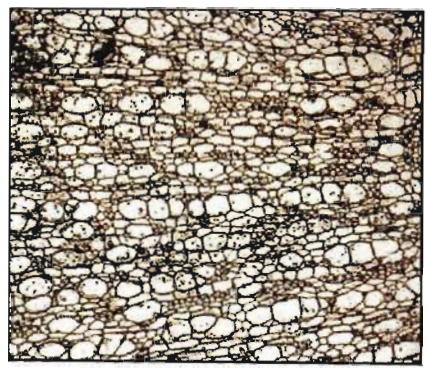
Control: *Barringtonia*, stem cross section X 10

High CO_2 Normal O_2: *Harpephyllym*, stem cross section X 10

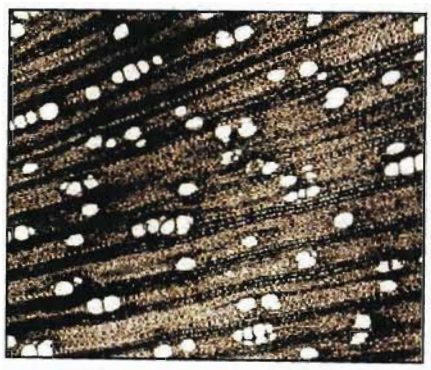


High CO_2 Normal O_2: *Barringtonia*, stem cross section X 10

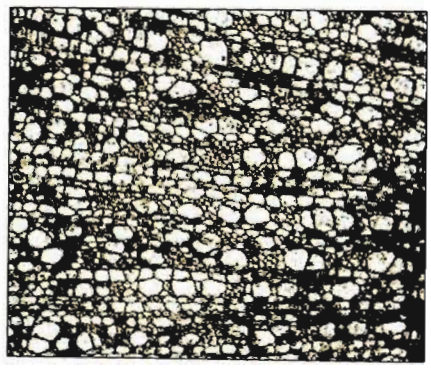
High CO_2 Low O_2: *Harpephyllym*, stem cross section X 10



High CO_2 Low O_2: *Barringtonia*, stem cross section X 10



Normal CO_2 Low O_2: *Harpephyllym*, stem cross section X 10



Normal CO_2 Low O_2: *Barringtonia*, stem cross section X 10

Figure 5.35: Cross sections of *Harpephyllum* and *Barringtonia* stem tissue showing no change in wood anatomy with treatment.

5.5 DISCUSSION

There were no mortalities or serious stress symptoms, such as epinasty or increased leaf loss, during the 140-day experiment, suggesting that elevated CO_2 (25%) and low O_2 (4%) concentrations used in this experiment do not cause an acute stress response. In terms of the physiological measurements made the response of both species to the treatments was only observed after approximately 75 days. This highlighted the importance of relatively long term investigations into the impact of landfill gas on plant species. It was also important that the plants were not killed by the treatments such that a detailed assessment of the plants functional response to the experimental conditions could be measured.

Since the early work of Leone *et al* (1977) it has been well documented that landfill gas pollution of the soil has a negative impact on plants. In accordance with previous observations the simulated landfill gas (i.e. elevated CO_2 low O_2 treatment) used in this experiment influenced the growth of both species, especially root growth. Similar simulated landfill gas experiments by Chan *et al* (1991) and Marchiol *et al* (2000) showed a reduction in plant root growth that was attributed to landfill gas induced stress. The observed reduction in root growth was not isolated to simulation experiments. The trees planted on the Bisasar Road landfill were exposed to high landfill gas concentrations and showed reduced rooting density (Chapter 4). Reduced root growth and has also been observed for trees planted at Edgeboro Landfill, New Jersey, where a significant negative correlation between high CO_2, low O_2 and total root length was reported (Gilman *et al* 1981).

The assessment of the relative effects of the separate components of the simulated landfill gas (i.e. normal CO_2 low O_2 and high CO_2 normal O_2 treatments) on root growth showed that high soil CO_2 (25%) despite good soil O_2 (20%) limited root growth for both species. Similarly Huang *et al* (1997) also found elevated soil CO_2 even with normal soil O_2 levels reduced root growth in both flooding sensitive and non-sensitive plants. Highlighting the distinct role that soil CO_2 can potentially have on plant growth. Elevated soil CO_2 appears to inhibit root respiration and modify carbon allocation (Conlin & van den Driessche, 2000; Nobel & Palta, 1989). This may be due to a decrease in cytosolic pH caused by soil CO_2 entering the cells by a hydration reaction (Nobel, 1990). Carbon dioxide levels in the root atmosphere ranging from 0.7% to 6.5% have been reported to cause inhibition of root respiration and growth in a number of different plant species by a number of researchers since as early as 1957 (Conlin & van den Driessche, 2000; Nobel, 1990; Nobel & Palta, 1989; Qi *et al*1994; Radin & Loomis, 1969; Stolwijk & Thimann, 1957). Therefore, the level of soil CO_2 (25%) used in this study was likely to have had an inhibitory effect on root respiration, thus explaining reduced root mass. Considering, a soil CO_2 level of 25% is not uncommon in landfill cover soils, reduced root respiration and growth is likely to be key factors influencing plant performance.

The relative effects of low soil O_2 (i.e. normal CO_2 low O_2) on root growth of *Barringtonia* was minimal, suggesting that reduced root mass seen in the simulated landfill gas treatment (high CO_2 low O_2) was primarily due to CO_2 (Figure 5.12). However, for *Harpephyllum* the root mass was reduced by low soil O_2 even in the presence of normal soil CO_2 levels (Figure 5.13). This is not unusual, as inhibition of root growth in response to soil oxygen deficiency has been reported for many vascular plant species (Kludze *et al* 1994). This reduced root growth is primarily due to inhibition of respiration and lack of an

electron acceptor, thus a shortage of ATP (Nobel & Palta, 1989). This can disturb the functional relationship between organs such as the roots and shoots (Drew, 1997; Vartapetian & Jackson, 1997). Generally soil O_2 levels below 10% restrict root growth and below 5% root growth ceases (Kozlowski, 1991). Considering the low oxygen treatments had mean O_2 levels between 3-5% the reduced root mass of *Harpephyllum* could be expected. It is apparent that the low soil O_2 conditions, like high soil CO_2, can have an inhibitory effect on root respiration. However, the fact that *Barringtonia* root mass was not influenced by the low soil O_2 conditions suggests that this species had mechanisms to manage the low soil O_2 conditions. These possible mechanisms will be considered when the root morphology and anatomy results are discussed later.

It is not uncommon for woody plants to experience a change in the rate of stem diameter growth in soils that become poorly aerated (Kozlowski, 1986). This is probably related to a shift in carbohydrate partitioning in response to root stress, causing changes in the growth rates of phloem parenchyma (i.e. increase in bark) and / or the number and size of xylem cells in the stem (Kozlowski, 1997). The reason or benefits of this response are unclear, and the response varies between species from temporarily or sustained accelerated growth rates to reduced growth (Kozlowski, 1986). This may provide an explanation for the variations in stem diameter growth, seen in this experiment, suggesting that the root stress created by the treatments was causing changes in the carbohydrate partitioning of the plants. *Harpephyllum* clearly showed a temporary increase in stem diameter growth in the high CO_2 normal O_2 and normal CO_2 low O_2 treatments (Figure 5.18). Whilst *Barringtonia* showed a temporary increase in the normal CO_2 low O_2 treatment and a more gradual but sustained higher rate of stem diameter growth in the treatments with high CO_2 (Figure 5.17). However, *Harpephyllum* showed little change in the rate of stem diameter growth in

the high CO_2 low O_2 treatment (Figure 5.18). Considering this treatment was also causing root stress, as seen by the reduced root mass (Figure 5.13), one would expect a shift in carbohydrate partitioning and a resultant change in stem diameter growth rate as seen in the other treatments. It was apparent that the combination of high CO_2 and low O_2 (simulated landfill gas) may be inhibiting a change in carbohydrate partitioning that was apparently induced by root stress in the other treatments.

When roots are depleted of O_2 it is important that the shoots respond metabolically to the root conditions and curtail their demand for root derived resources (Vartapetian & Jackson, 1997). The physiological measurements indicated that there was reduced metabolic activity of *Barringtonia* shoots in response to the normal CO_2 low O_2 treatment. The stomatal conductance of *Barringtonia* in the normal CO_2 low O_2 treatment was usually the lowest of the treatments from the first week of the experiment and after 111 days it was consistently and significantly ($p<0.05$) lower than the control. Under low soil O_2 conditions stomatal closure is possibly more than a passive response to poor water absorption by an energy deficient root system (Sojka & Stolzy, 1980). Jackson, (1994) and Smit *et al* (1990) suggested that the hypoxic status of roots is transmitted by an unknown signal in the transpiration stream resulting in a metabolic response in the leaves. The closure of the stomata and resultant low stomatal conductance under low soil oxygen conditions causes stomatal limitation of the photosynthetic system (Kludze *et al* 1994, Pezeshki *et al* 1996). This is not an uncommon phenomena, as stomata have an integral role in the regulation and control of photosynthesis (Jones, 1998). The depression of carbon assimilation and photosynthate utilization is an important response for maintaining the functional relationship between the roots and shoots and the 'management' of low O_2 soil conditions (Vartapetian & Jackson, 1997). The light and Aci response measurements clearly showed

lower carbon assimilation rates of *Barringtonia* in the normal CO_2 low O_2 treatment after 140 days. The apparent down regulation of leaf carbon assimilation would curtail shoot demands on root activity allowing for a functional equilibrium within the plant to be maintained and continued root function and growth. The reduced demand from the shoots for resources from the root would allow for root growth to be maintained, as illustrated by the lack of significant difference in the root mass between the control and the normal CO_2 low O_2 treatment (Figure 5.12).

Unlike *Barringtonia, Harpephyllum* in the normal CO_2 low O_2 treatment showed no evidence of stomatal limitation and reduced carbon assimilation in the shoots (Figure 5.22 and 5.23 respectively), however, root growth was significantly reduced. There was no evidence suggesting that shoot demand on the roots was alleviated which could have resulted in an imbalance in the functional equilibrium of the plant. If there were no means to alleviate the oxygen stress on the roots, the continued demand for resources by the shoots would result in reduced root growth, as seen by the reduced root mass (Figure 5.13). It is proposed with a longer experimental period (>140 days) a further deterioration of root growth and function would be observed and reduced stomatal conductance and carbon assimilation rates would inevitably occur due to photosynthetic system failure and not controlled down regulation.

Arthur *et al* (1981) found that the stomatal conductance of a tree species (*Acer saccharum*) with a known sensitivity to landfill soil conditions was significantly reduced by simulated landfill gas (3% O_2; 40% CO_2; 50% CH_4; 7% N_2). A field investigation by Gilman *et al* (1989) also showed that the elevated CO_2 and low O_2 conditions in landfill cover soils were associated with reduced stomatal conductance and lower plant growth in *Acer*

saccharum. Similarly in this study, *Harpephyllum caffrum*, a landfill sensitive species, showed a reduced stomatal conductance when the roots were fumigated with simulated landfill gas (i.e. high CO_2 low O_2). However, the results of this study suggested that the primary cause of reduced stomatal conductance of *Harpephyllum* was probably the high soil CO_2 and not low O_2. This was concluded because, unlike the normal CO_2 low O_2 treatment, the high soil CO_2 with or without low O_2 resulted in a significant reduction in stomatal conductance after only 75 days of fumigation and a reduced maximum carbon assimilation rate in the light response curve after 140 days. However, root growth was also significantly reduced by the treatments with elevated CO_2. The reduced root growth, indicated that the lower stomatal conductance and carbon assimilation rates were probably less likely to be a controlled down regulation of shoot demand on roots, as seen for *Barringtonia* in the normal CO_2 low O_2 treatment, and more likely a symptom of photosynthetic system failure. Considering that stomatal closure is usually one of the first responses to root stress (Liang *et al* 1995), it is possible that the physiological response of *Harpephyllum* to the elevated CO_2 treatment was due to CO_2 damage to root cells. In fact, the reduction in stomatal conductance became so severe that there was insufficient leaf – atmosphere gas exchange towards the end of the experiment for further measurements to be conducted. It was apparent that for *Harpephyllum*, high soil CO_2 possibly had a more rapid and marked effect than low soil O_2 on root function, resulting in a relatively more rapid limitation of carbon assimilation by shoots.

Further evidence that that high soil CO_2 had a more marked effect on *Harpephyllum* root function than low soil O_2 was provided by the leaf nutrient analysis. The results suggested elevated CO_2 and not low O_2 was causing lower leaf nutrient content, especially for K, Mn, Cu, Mg and P (Table 5.3). This indicated that high soil CO_2 was resulting in limited

nutrient uptake and / or transport to the shoots. This is not an uncommon phenomenon and Chang & Loomis (1945) showed reduced root uptake of nutrients due to elevated CO_2 in the root zone over 50 years ago. It was also noted by Ruark *et al* 1982 who attributed the lower nutrient uptake of roots to carbon dioxide toxicity, which decreased root permeability. In fact low O_2 appeared to have an antagonistic impact on the lower leaf nutrient content in *Harpephyllum* caused by elevated CO_2 (Table 5.3).

Although the elevated CO_2 treatments (high CO_2 low O_2 and high CO_2 normal O_2) also resulted in reduced root mass of *Barringtonia* the response of the species was different to *Harpephyllum*. Unlike *Harpephyllum* the high soil CO_2 conditions showed no clear impact on nutrient uptake. Also unlike, *Harpephyllum* the stomatal conductance and maximum assimilation rates in the light and Aci response curves did not vary significantly from the control after 140 days of the elevated CO_2 treatment. Similarly, Arthur *et al* (1981) found that stomatal conductance of a tree species (*Acer rubrum*) with a known 'tolerance' to landfill conditions was not affected by simulated landfill gas (3% O_2; 40% CO_2; 50% CH_4; 7% N_2). However, in this study it was also apparent that the elevated CO_2 was ameliorating the depression of stomatal conductance and carbon assimilation that was caused by the low soil oxygen conditions. The amelioration by elevated soil CO_2 of photosynthetic system depression caused by low soil O_2 has been observed, especially in species that are flooding tolerant (Huang *et al* 1997). However, the mechanism is not clear but several possibilities have been proposed such as changes in leaf ribulose–1,2-bisphosphate carboxylase-oxygenase or transport of root CO_2 through aerenchyma to shoots or the counter effects of CO_2 on ethylene inhibition (Huang *et al* 1997). These are discussed further below.

It was proposed by Arteca and Poovaiah, (1982) that Ribulose –1,2-bisphosphate could make use of CO_2 translocated from the roots to suppress photorespiration. This would reduce CO_2 production by respiration and result in higher apparent carbon assimilation as measured by leaf – atmosphere exchange in light and Aci response curves. The movement of CO_2 or other gases such as methane from the root to the shoot through aerenchyma has been demonstrated recently (Jackson & Armstrong, 1999; Le Mer & Roger 2001), thus the theory is not unreasonable. Arteca and Poovaiah, (1982) also showed that root zone application of CO_2 enhanced phosphoenolpyruvate carboxylase activity in the roots of some species, which can facilitate the fixing of root zone CO_2 into malate. This was confirmed by Gao and Lips, (1997) and they further showed that the malate produced was important for respiratory energy function and resulted in increased NO_3^- uptake. The increased NO_3^- was found to stimulate the transport of carbon assimilates to the shoot. Thus, it is possible that elevated root zone CO_2 was ameliorating the effects of low O_2 on root respiration allowing for a functional relationship with the shoots to be maintained.

The effects of ethylene inhibition by CO_2 is also a possible explanation for apparent ameliorative effects of elevated CO_2 on the impact of low O_2 seen in *Barringtonia*. Ethylene production by roots and soil micro-organisms is a common response to low levels of soil O_2, however high concentrations of ethylene may reach leaves via intercellular spaces affecting leaf physiology (Jackson *et al* 1987). Carbon dioxide has been shown to have an inhibitory effect on the influence of ethylene on plant metabolism (de Wild *et al* 2002; Radin & Loomis, 1969), thus it is also possible that elevated CO_2 reached the leaves via aerenchyma and prevented the impact of ethylene. It is not possible in this experiment to identify which mechanisms, if any, allowed *Barringtonia* to use CO_2 to escape the impact of low O_2 on shoot physiology. However, it is clear that aerenchyma as well as

enhanced enzyme activity within the plant could be of distinctive advantage for survival and growth in elevated CO_2 low O_2 soil environments such as those found in landfill cover soils.

A greater understanding of the mechanisms of root survival and growth in landfill soils was provided by the assessment of root morphology in this experiment. Both species showed a significant reversal in rooting depth gradient and a higher proportion of roots near the soil surface in the simulated landfill gas treatment (high CO_2 low O_2) relative to the control. However, it is important to note that the closed chamber design did not allow for atmospheric dilution of the gas treatment near the soil surface therefore, unlike in landfill cover soils in the field, shallower rooting would not allow avoidance of the high CO_2 and low O_2 conditions. There was also unlikely to be a soil moisture gradient within the chamber as loss of water through evaporation from the soil surface would also be minimal due to the closed chamber design. This suggests that in this fumigation experiment the shallower rooting was not simply a response to an environmental gradient within the chamber soil, but a distinct plant response to the soil atmosphere conditions. Interestingly, the results suggested that in *Harpephyllum* shallower rooting was primarily driven by the high soil CO_2, whilst in *Barringtonia* low soil O_2 appeared to be the key factor. Elevated soil CO_2 and not low O_2 has been reported as the main cause of shallower root growth for plants with normal sensitivities to CO_2 and O_2 in landfill cover soils (Chan *et al* 1991; Gilman *et al* 1981). This indicated that *Barringtonia* was unlike most other species and avoidance of high soil CO_2 did not appear to be of primary importance in its rooting response in this experiment. This concurred with the physiological results that showed little effect of high soil CO_2 on *Barringtonia* physiology.

Leone *et al* (1983) screened 19 tree species for landfill tolerance on a New Jersey landfill, the results indicated that the relatively 'tolerant' species had shallower rooting depths than less 'tolerant' species. Based on this research and other similar experiments it has been suggested that the ability to develop a shallow root system and avoid the high CO_2 and low O_2 conditions found deeper in the soil is a critical factor in determining the survival of trees on landfills (Gilman *et al* 1982 and 1981; Leone *et al* 1983). It has also been noted that some species with inherent shallower rooting (i.e. even under normal soil conditions) perform better on landfills (Gilman, 1989; Leone *et al* 1983).

The fundamental question is, if for both species the simulated landfill gas resulted in a distinct shallower rooting response that is characteristic of tolerant species how does *Barringtonia* maintain better root function? This question is not restricted to these species or this experiment. Gilman *et al* (1982) showed that Hybrid Poplar (*Populus* spp) and Green Ash (*Fraxinus lanceolata*) both show distinct shallower rooting response to landfill conditions. However their experiment, and the work by Leone and Flower (1982), indicated that Poplar has a greater ability to maintain growth and survival on landfills than Green Ash. It was also observed by Chan *et al* (1991) that the resultant shallower rooting depth in the landfill cover soil also made tree species more susceptible to water stress. Thus it appears that the ability to develop a shallow root system has both advantages and disadvantages especially in dry seasoned climates, and other mechanisms are clearly of use in maintaining root survival and growth under elevated soil CO_2 and low soil O_2 conditions.

The fact that shallower rooting is a common response of plants growing in soils contaminated with landfill gas clearly indicates that most plants try to avoid the resultant

high soil CO_2 and low O_2. However, the ability to maintain a functional root system when landfill gas is unavoidable, like *Barringtonia* in this study, is also clearly beneficial. *Barringtonia* should perform better in soils where there is little atmospheric dilution of landfill gas in surface soil and / or low moisture in the surface soils making shallower rooting of little benefit. The results of this experiment suggest that the key to *Barringtonia*'s ability to maintain root functionality in the unavoidable simulated landfill gas treatment was related to the anatomy of the roots and stem of the species. Unlike *Harpephyllum*, *Barringtonia* had anatomical features which were characteristic of a flood tolerant species. The similarity between flooded and landfill soil atmospheres has commonly lead to the proposal that species adapted to flooding are potentially suitable for planting on landfills (Gilman *et al* 1985; Leone *et al* 1977; Zhang *et al* 1995). It has also been experimentally shown that flooding- tolerant species tend to be more tolerant of landfill gas than flooding- sensitive species (Arthur *et al* 1981). However, little research into the actual anatomical characteristics, which facilitate better performance under landfill gas fumigation, has been conducted.

The most widespread anatomical feature conferring tolerance of flooded soils is an interconnected system of gas spaces (aerenchyma) within the plant (Jackson, 1994). There was clear evidence of lysogenous aerenchyma in the roots of *Barringtonia*, unlike *Harpephyllum* which had very little intercellular root airspace. Aerenchyma is formed either by cell wall separation without collapse, forming a honeycomb appearance of the root cortex (schizogeny) or, as in this case, by programmed cell collapse resulting, normally, in radial air spaces in the cortex (lysogeny) (Jackson & Armstrong, 1999; Laan *et al* 1989). In both *Harpephyllum* and *Barringtonia* there was no apparent effect of the experimental treatments on tissue anatomy and the aerenchyma in the *Barringtonia* roots

was constitutive, which is often a characteristic of flood tolerant species (Drew, 1997). The root aerenchyma in *Barringtonia* would have provided a lower number of energy demanding cortical cells requiring oxygen and formed an internal pathway of high conductivity for gases, thus enhancing internal oxygen diffusion. The ability of aerenchyma tissue to transport oxygen from the shoots to the roots has been shown by experimentally (Jackson & Attwood, 1996; Kludze *et al* 1994; Wiedenroth & Erdmann, 1989). It is also apparent that the mass flow of gas through aerenchyma is unnecessary, as molecular diffusion of oxygen is sufficient to supply root cell respiration, thus making shoot–root oxygen exchange more plausible (Moog & Bruggemann, 1998). Aerenchyma also enhances radial oxygen diffusion allowing gas phase oxygen transport from the central core of the root (Veen, 1987, Wiedenroth, 1993). Efficient radial oxygen diffusion is also important because it also allows for easy movement of oxygen from outside the root through to the central core, as well as easy movement of oxygen within the root. This can increase the availability of the minimal oxygen present within the surrounding soil and within the root. Thus providing the oxygen required for maintenance of nutrient uptake and importantly transportation to the shoots, as illustrated by Topa & Cheeseman, (1994) with their work on *Pinus serotina* under hypoxic growth conditions.

The availability of oxygen to the root cells is also largely dependent on the cell configuration and the degree of secondary thickening. The cortical cells of *Barringtonia* showed a cubic packing arrangement forming concave quadrangulus intercellular air spaces. This cell arrangement was described by Justin and Armstrong, (1987) who , in a study of 91 plant species, identified it as providing maximum gas space per unit tissue volume and the most appropriate cell configuration for plants that rely upon internal ventilation for root aeration. Unlike *Barringtonia*, *Harpephyllum* showed a dense cell

arrangement and very little intercellular airspace. *Harpephyllum* also did not maintain an apparent juvenile root structure like *Barringtonia* but had a high degree of secondary thickening. Secondary thickening rapidly destroys the primary cortex and any primary aerenchyma that may have formed, it also decreases intercellular air space and the potential for internal ventilation (Jackson & Armstrong, 1999; Moog, 1998). Thus it was clear that relative to *Harpephyllum, Barringtonia* had the better root cell configuration to make optimum use of minimal oxygen and allow for maximum internal ventilation.

There were clear differences in stem anatomy between the species, although there was no aerenchyma tissue present in either species, *Barringtonia* had a distinctly more open wood structure and large fibre cells which would be more conducive to internal ventilation. Porosity measurements confirmed that *Barringtonia* stems had a significantly greater amount of airspace than *Harpephyllum* stems. In fact porosity measurements based on Archimedes principle can be up to 60% more accurate than microscopic sections which can overestimate porosity by including all spaces between cells which are not all gas filled (Jackson & Attwood, 1996). High root tissue porosity values are also indicative of the presence of aerenchyma tissue and confirm that intercellular spaces are gas filled (Connel *et al* 1999, Kludze *et al* 1994; Van Noordwijk & Brouwer, 1988). Thus the porosity measurements confirmed the observations and conclusions reached from the root and stem microscopical cross sections. However, they also provided a clear quantitative indication of the difference in anatomy between the two species. *Barringtonia* had mean root and stem porosity values in the simulated landfill gas treatment that were in excess of 9% whilst *Harpephyllum* had significantly lower values that were less than 1.5%. Porosity values less than 7% are found in species that are sensitive to flooded soils (Justin & Armstrong, 1987) and values between 3-5% were associated with very low rates of internal

oxygen diffusion and were responsible for restricted root growth in anaerobic soils (Voesenek *et al* 1999). This provides a clear reason why *Harpephyllum* was unable to maintain root functionality whilst *Barringtonia* could. The lack of internal ventilation within *Harpephyllum* probably resulted in insufficient oxygen availability to the root cells thus reducing nutrient uptake and transport to the shoots. It was apparent that anatomical characteristics associated with internal tissue ventilation were important for better performance under elevated CO_2 and low O_2 conditions and confirmed that characteristics usually associated with flood tolerant species are an important consideration in selecting species for landfills.

In conclusion the results for growth, physiology, and leaf nutrients confirm the hypothesis that the impact of elevated CO_2 and low O_2 is greater on *Harpephyllum* than *Barringtonia*. This reinforced the premise that landfill gas was the key cause for differential performance of these species on the landfill. The results indicated that the key impact of landfill gas was on root system function and the functional relationship between roots and shoots. It was also clear that the roots of both species would prefer to avoid the landfill gas soil conditions, however, this is not always possible or beneficial thus internal tissue ventilation was identified as the key characteristic associated with *Barringtonia* success in an unavoidable landfill gas saturated soil. Elevated CO_2 appears to cause direct toxicity effects on roots which enhances the negative effects of low O_2 on a sensitive species like *Harpephyllum*. However, *Barringtonia* appears to have mechanisms, possibly related to root enzyme activity and aerenchyma tissue, which prevent the negative effects of CO_2 and even make use of CO_2 to reduce the impact of low O_2 on root respiration.

CHAPTER 6: FINAL DISCUSSION

6.1 ENVIRONMENTAL VARIABLES LIMITING VEGETATION GROWTH IN A LANDFILL ENVIRONMENT

In order to improve the stability and aesthetics of operational landfills and increase the scope of rehabilitation planning for closed landfills, successful vegetation establishment is clearly advantageous. Operational sites are permanently undergoing landscape changes in order to accommodate incoming wastes and reduce erosion. Tree damage is not uncommon during construction activity and the value of plants financially and in terms of environmental benefits is not always considered (Yingling *et al* 1979). However, careful planning of site operations and forethought before any revegetation or site construction can easily remedy the impact of earth moving machinery. A more difficult problem to address is the unique and harsh combination of environmental soil variables that challenge plant survival and growth on landfills. The investigations and experiments conducted on the Bisasar Road landfill confirmed the work of others showing that the landfill environments are a formidable challenge to vegetation growth, especially for trees (Chan *et al* 1991; Lan & Wong 1994; Dobson & Moffat 1994; Ettala *et al* 1988; Flower *et al* 1981; Gilman *et al* 1981).

In order to achieve successful revegetation a thorough understanding of the environmental conditions limiting plant growth is essential. The research on the Bisasar Road Landfill highlighted several soil variables that were primarily responsible for poor grass coverage and tree survival and growth. In summary, the results highlighted the importance of soil CO_2 in determining the performance of plants on landfills. However, the compounding effects of other environmental variables such as low soil O_2; changes in soil redox potential; low soil moisture; and high soil conductivity were also identified as potentially

critical variables in determining overall success of a plant. A simple model of the key variables possibly responsible for poor vegetation growth on the Bisasar Road Landfill is provided in Figure 6.1.

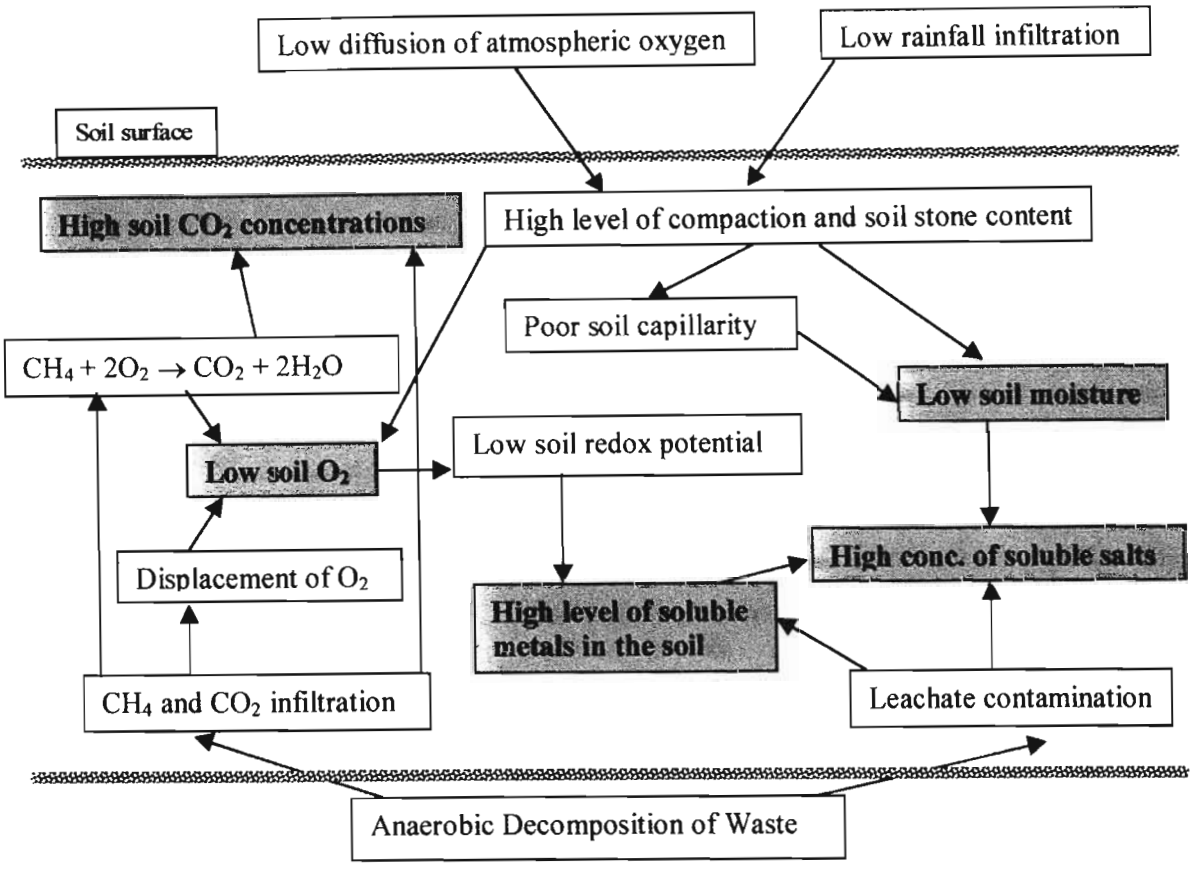


Figure 6.1: A simple model of the inter-relationship of variables that produce the 5 main variables likely to limit plant growth on landfills.

All three studies on the Bisasar Road landfill suggested that landfill gas infiltration into the root zone was the main factor limiting the growth and survival of plants on the landfill. The difference in performance of *Barringtonia racemosa* and *Harpephyllum caffrum* on the landfill (Chapter 4) was similar to that in the simulated landfill gas experiment (Chapter 5), further supporting the premise that landfill gas was the key cause for poor performance of plants on the landfill. The identification of landfill gas as a main factor

limiting plant growth and survival on the Bisasar road landfill is a common conclusion (e.g. Leone & Flower, 1982).

Further, the results of this research provided evidence to support the theory suggested by others that elevated soil CO_2 was the main constituent of landfill gas influencing plant survival and growth on landfills (Chan *et al* 1991, Gilman *et al* 1981, Leone *et al* 1977). A brief summary of the evidence follows. In the first investigation elevated soil carbon dioxide was associated with poor grass colonisation, even though the soil was aerobic (see Chapter 2). The field investigation into tree mortality showed poor tree health was associated with high soil methane and carbon dioxide (Chapter 3). The tree field experiment on the landfill also provided results that suggested that soil CO_2 levels had a key role in influencing plant health (Chapter 4). Further confirmation of the importance of soil CO_2 levels was provided by the fumigation experiment that showed a clear negative effect of elevated CO_2 with or without normal soil O_2 on the physiology and growth of a landfill sensitive species, *Harpephyllum caffrum* (Chapter 5).

However, the experimental evidence suggests that the role of low soil O_2, caused by displacement of soil air by landfill gas and by methane oxidation (Figure 6.1), also needed consideration. Low soil oxygen alone (i.e. without elevated CO_2) can have a negative effect on plant physiology and the root growth of most plants (Huang *et al* 1997, Jackson & Armstrong, 1999). Similar to the findings of Huang *et al* (1997) the fumigation experiment showed that low soil O_2 can make the impact of elevated soil CO_2 more pronounced, especially for CO_2 sensitive species such as *Harpephyllum caffrum.* However, it must be noted that the response of *Barringtonia racemosa* in the fumigation experiment clearly showed that there are possible mechanisms that allow some species to

avoid the negative effects of both low O_2 and elevated soil CO_2. The general performance of *Barringtonia racemosa* was better than that of most other species and the possible mechanisms allowing this will be discussed further below (Section 6.2).

The results of this study provide an opportunity for the evaluation of threshold levels of soil CO_2 and O_2 that are likely to be problematic for plants on landfills. The colonisation of grass appeared to be limited by a root zone CO_2 level of about 14%, even with a relatively aerobic soil of about 12% O_2. However, the soil gas concentrations were not quantified over an extended period of time therefore sporadic or episodic pulses of higher soil CO_2 cannot be discounted as the possible cause for poor grass colonisation. An evaluation of the literature (in Chapter 4) indicated that CO_2 levels of 14% can be associated with poor plant growth and high mortality. Recently, Marchiol *et al* (2000) also found that a simulated landfill gas containing 16% O_2, 8% CO_2 and 3% CH_4 caused a delay in seed germination of a number of plant species. Therefore a CO_2 level of 14% and O_2 of 12% could possibly act similarly and delay or even prevent seed germination. Thus, the CO_2 and O_2 values recorded in the bare areas provided a reasonable explanation for a lack of grass. However, there may also have been additive effects of other adverse environmental conditions, such as soil moisture limitations, albeit these conditions could have been a resultant effect of the lack of grass cover, initially caused by soil gas conditions, but they could then subsequently limit further plant colonisation.

In the field experiment assessing tree performance on the landfill (Chapter 4), the same topsoil was used on the control site and on the landfill. A comparison of the soil variables between the topsoil on the control and that on the landfill during the experiment, provided an indication of the changes the landfill environment can have on soil quality. The topsoil

on the landfill was found to have lower soil moisture and soil O_2, and higher soil CO_2 and extractable Mn in comparison to the topsoil on the control site. Although the change in Mn was considered a possible indicator of soil quality deterioration it was considered unlikely to be problematic for the trees during the experiment and will be discussed later. However, the trees planted on the landfill plot with a topsoil layer still experienced a relatively high level of mortality (24%) during the 435 day experiment. Based on the changes in the topsoil variables, the most likely soil variables responsible for the mortalities were soil moisture, soil O_2 and soil CO_2. The analysis of rooting depth indicated that roots were restricted to a soil depth at which CO_2 levels were less than 20-27% and O_2 levels were greater than 1-2%. Considering it was shown that the CO_2 levels decreased and O_2 levels increased towards the soil surface, the majority of the tree root systems on the experimental landfill plots were probably exposed to less extreme soil gas conditions. In order to consider further the concentration thresholds for soil CO_2 and O_2 and plant response the discussion will focus on *Harpephyllum caffrum*. This species appeared to be sensitive to the landfill environment and its response to elevated soil CO_2 and low soil O_2 were assessed in both the field and fumigation experiment.

In the field experiment *Harpephyllum caffrum* experienced 57% mortality on the landfill topsoil plot within 187 days with the mean CO_2 of 25% and mean O_2 level of 3%. However, in the fumigation experiment, which exposed *Harpephyllum caffrum* roots to similar CO_2 (25%) and soil O_2 (5%) concentrations to the field experiment, a slow deterioration of health but no mortalities during the 140 day experiment was observed. Although mortalities were likely in the long term, the difference in the duration of the two experiments (47 days) was unlikely to completely explain the higher mortality seen in the field experiment. It must be acknowledged that the fumigation experiment was based in a

greenhouse that would have provided optimum growth conditions, and the plants were regularly watered. Therefore the mortality of *Harpephyllum* trees in a relatively shorter time period on the landfill was probably due to the negative additive effects of other environmental stresses found in the field. Figure 6.1 provided a summary of some of the possible below ground variables, however, important above ground variables could include increased stress due to high winds, dust, and possibly air pollution.

One of the key variables that may influence the severity of the effects of soil CO_2 is available soil moisture (Figure 6.1). Low soil moisture was correlated with poor grass colonisation and poor survival of some trees in the field investigations. The application of topsoil over the cover material was found to improve soil moisture levels and also tree survival and growth in the field experiment. Improved soil moisture conditions are usually associated with better soil structure, as was provided by the topsoil layer. However, the quality of cover material used on landfills is usually poor due to availability and high cost of good quality topsoil (Flower, *et al* 1981). High stone content can reduce soil capillarity, thus reducing the upward migration of moisture (Heilmann, 1981; Insley & Carnell, 1982). Soil moisture levels are further limited by the practice of compacting cover soils (Butt *et al* 1999; Flower *et al* 1981; Greacen & Sands, 1980), in order to reduce water infiltration which causes leachate production (Cooper *et al* 1997), and to maximise fill space (Flower, *et al* 1981). Therefore, the low soil moisture levels found on the Bisasar Road Landfill were not unusual and it is not surprising that low soil moisture has been highlighted as a problem for plant growth on landfills (Gendebien, *et al* 1992; Liang *et al* 1999). It is important to note that low soil moisture problems on landfills are particularly problematic in areas that receive relatively low and seasonal rainfall such as in the Bisasar Road Landfill in Durban and in most of southern Africa (Chapter 1, Table 2.1).

Low soil moisture conditions can also compound the effects of other variables such as the high concentrations of soluble salts in the soil. The soil conductivity levels in the landfill cover material were in excess of the minimum standards for woodland establishment (Moffat & Bending, 1992) and in conjunction with low moisture availability the potential for osmotic and ionic stress on the vegetation becomes more severe (Bradshaw & Chadwick, 1980). Although this is a potential problem the investigation of grass growth indicated that the natural colonisers of the site were generally tolerant of high soil conductivity. Therefore, it was not the key reason for patchy grass growth. However, the trees generally responded well to the topsoil layer, which had a significantly lower soil conductivity and higher soil moisture levels, suggesting that on the poor landfill cover soils the level of soluble salts in the soil may have had an impact on tree growth and survival. Thus the relatively high conductivity of the soil in conjunction with the low soil moisture conditions were likely variables responsible for enhancing the severity of the effects of high soil CO_2.

High concentrations of soluble salts in landfill soils are generally caused by leachate contamination (Dobson & Moffat, 1994; Lan & Wong, 1994; Menser *et al* 1983; Wong *et al* 1992). The relatively high levels of soil Ca found in the grass field investigation and the field tree experiment can be indicative of leachate contamination of landfill cover material (Hernandez *et al* 1999). Further evidence, such as relatively high soil pH, and high K concentrations found, also suggested that the cover material on the landfill maybe contaminated with leachate (Winant *et al* 1981).

Heavy metal (Tong & Wong, 1984) and possibly chloride contamination of the soil, due to leachate, may result in phytotoxity (Menser *et al* 1983; Ettala, 1988). However, analysis of

the total metal content of the landfill cover material and the additional topsoil layer during the field experiment showed that levels of metal contamination of the soil was minimal and unlikely to be phytotoxic. The concentration of heavy metals in leachate from landfills is generally low and does not usually constitute a significant pollution problem (Christensen *et al* 2001). Therefore, metal toxicity was an unlikely reason for poor plant growth and survival on the landfill. Leachate, with high concentrations of ions, can cause changes in the soil chemistry, sometimes resulting in the leaching of soil nutrients (Dobson & Moffat, 1994). This may provide an explanation for the low Mg concentrations measured in the landfill cover material (Chapter 4). It was apparent that leachate contamination of the landfill cover material can result in deterioration of soil quality. However, the evidence from the grass bioassay and the analysis of soil nutrient indicated that soil leachate contamination and the resultant change in soil nutrient content was minimal. Therefore, the influence of leachate was unlikely to have any great effect on plant growth and survival in this research.

An increase in extractable Mn seen in the topsoil placed on the landfill originally raised concerns about leachate contamination. However the lack of significant difference in total Mn concentrations between the experimental plots proved that Mn levels were not due to an external source of contamination. The increasing levels of Mn in the topsoil on the landfill was attributed to the low oxygen levels in the topsoil layer created strong reducing conditions which cause insoluble Mn^{4+} to form the highly soluble Mn^{2+}. This could possibly change the ratio of total manganese to ammonium bicarbonate EDTA extractable manganese (Crawford, 1989; Menser *et al* 1979; Munshower, 1994; Rees, 1982). Mn toxicity in plants is not uncommon, especially under strongly reducing conditions (Gonzalez & Lynch, 1999; Mgema & Clarke, 1995). Mn is usually found to accumulate in

the leaves resulting in a decline in photosynthetic activity by interfering with the activities of the CO_2 reduction cycle (Kitao *et al* 1997). Although net photosynthesis or leaf Mn levels were not measured in the field experiment, they were measured in the simulated landfill soil atmosphere experiment. Although simulated landfill conditions showed a reduction in the net photosynthesis for the landfill sensitive species (*Harpephyllum caffrum*) there was no evidence of elevated leaf Mn levels. It may be inferred from this that Mn toxicity was unlikely to be the cause of poor plant performance on the landfill. However, this is not conclusive and further field measurements that include soil redox potentials and leaf Mn levels would help confirm any detrimental effects of soil Mn.

It can be concluded that landfill gas infiltration into the root zone and the resultant elevated soil CO_2 conditions is the primary cause of poor plant growth on landfills. However, the severity of the effect is largely dependant on species tolerance and the compounding effects of other variables such as low soil oxygen, low soil moisture and possibly leachate contamination of the soil.

6.2 SPECIES TOLERANCE

In the natural environment low soil O_2 conditions are not uncommon. Nearly 6% of the Earth's surface is classified as wetland and is flooded for at least part of the year (Maltby, 1991), resulting in low soil oxygen conditions (Crawford, 1989). Therefore plants tolerant to low soil oxygen conditions, similar to that found on landfills, are not uncommon. *Barringtonia racemosa, Hibiscus tiliaceus* and *Combretum erythrophyllum,* which performed best on the landfill in this investigation, grow in natural habitats bordering swamps and river courses. Interestingly, the tree species that performed poorly on the landfill were associated with natural habitats that were unlikely to have waterlogged soils.

These species included *Erythrina lysistemon, Rhus lancea, Acacia sieberiana, Strelitzia nicolai,* and *Harpephyllum caffrum* (Palgrave, 1984; Pooley, 1994). This relationship between species from waterlogged habitats and tolerance to landfill conditions has been reported by a number of investigators (Arthur *et al* 1981; Chan *et al* 1991; Crook, 1992; Gilman *et al* 1985; Leone *et al* 1977). This apparent relationship is obviously unlikely to be associated with soil moisture similarities between the two habitats, as the landfill soil moisture levels can be relatively low (Crook, 1992; Gilman *et al* 1985). The relationship is attributed to the similarity in soil O_2 levels between waterlogged habitats and landfill cover soils (Arthur *et al* 1981; Gilman *et al* 1985).

However, it is important to note that not all waterlogged soils have low soil O_2. Turbulent flood waters often have sufficient oxygen in the water for aerobic respiration of roots (Gill,1970; Mckersie & Leshem, 1994). Therefore, one has to be more specific about the characteristics of the waterlogged habitat. Those characterised by more permanent and stagnant water, such as swamps and marshes, tend to have much lower soil oxygen levels (Mckersie & Lesham, 1994) and, therefore, more likely to be habitats with species tolerant of low soil O_2. It is also critical to consider the potential difference in soil moisture content between a waterlogged soil and a dry landfill cover soil. Low soil moisture conditions and low oxygen conditions seldom occur together in natural habitats. Species that inhabit areas with soils saturated with stagnant water during the wet season, but are also exposed to low soil moisture conditions in the dry season, maybe tolerant of low soil O_2 and low soil moisture conditions. This is the case for *Barringtonia racemosa* which has a natural distribution within swamp forest associated with rivers, estuaries or coastal areas, but grows well in wet and dry conditions (Palgrave, 1984; Pooley, 1994). It is rather unusual for a species to be tolerant of such a broad range of soil moisture conditions, however it is

probably one of the key reasons contributing to the good performance of *Barringtonia racemosa* on the landfill.

However, the natural habitat of a species is not always a clear guideline to the potential performance of a species in landfill environments. For example *Syzygium cordatum* is usually found on river banks (Palgrave, 1984; Pooley, 1994). Therefore, it would probably be exposed to waterlogged soils or at least to periods of waterlogging, however, this species was one of the most sensitive species to the landfill conditions. The results of the tree investigation (Chapter 3) indicated that the poor performance of *Syzygium cordatum* on the landfill was related to low soil moisture, which it may not experience in its natural habitat. It could also be attributed to river flood waters, as opposed to stagnant water, being relatively rich in O_2 therefore levels of soil O_2 may not be as low as landfill soils. Although these ideas are all speculative, it highlighted the difficulties in trying to correlate the similarities between a species natural habitat description and a landfill environment. It is apparent from this investigation and that by Arthur *et al* (1981) that it can sometimes provide an indication of species potential, however, the similarity between a waterlogged soil and a landfill cover soil is only apparent on a very simplistic level i.e. potentially low soil O_2. In waterlogged soils the prime cause of poor plant performance is the poor availability of O_2 for the roots (Jackson & Armstrong, 1999). However, this investigation and other landfill research indicate that the prime cause of poor performance of plants on landfills is elevated soil CO_2, to which low O_2 has an additive effect (Chan *et al* 1991; Gilman *et al* 1981, Leone *et al* 1977). Therefore the primary determinant of plant health and performance differs between waterlogged soils and landfill cover soils. It is also important to consider the difference in soil moisture between the two habitats.

It was apparent in both the field and fumigation experiment that tree roots of all species investigated tried to avoid high levels of soil CO_2 and low O_2 through shallower rooting. However, the severity of this response was more marked in those species that performed poorly on the landfill and the response appeared to be mainly driven by elevated soil CO_2 and not low O_2. The conclusion that soil CO_2 was the driving force behind shallower rooting depths on landfills has been reached by others (Chan *et al* 1991; Gilman *et al* 1981). However shallower rooting has previously been considered a response that is beneficial for survival on landfills, as it allows for the avoidance of adverse soil atmosphere conditions found deeper within the soil (Gilman *et al* 1982; Gilman *et al* 1981; Leone *et al* 1983). It is clear that shallower rooting can allow for avoidance of poor soil atmosphere conditions, however, as suggested by Chan *et al* (1991), it results in a greater susceptibility of plants to water stress, especially in arid climates and where there are low soil moisture levels. Therefore species performance on landfills, as indicated by the root morphology results of this study, is more likely to be associated with ability of species to maintain relatively deeper rooting despite the poor soil atmosphere conditions.

The ability to maintain a functional root system in the presence of elevated soil CO_2 is critical to achieving greater rooting depth. The results of the fumigation experiment indicated that for *Barringtonia racemosa*, this ability is closely related to an inherent specialised tissue arrangement of the roots and shoots, increasing intercellular airspace. This species also appears to maintain health under poor soil atmosphere conditions through the control of the resource demands of shoot and root through an unknown mechanism involving the avoidance of CO_2 toxicity, which may involve the transport and leaf utilisation of soil CO_2 to it own metabolic advantage.

In comparing the characteristics of *Barringtonia racemosa*, a species that performed well on the landfill, with that of *Harpephyllum caffrum*, a species that performed poorly, some of the possible mechanisms that allowed better species performance have been elucidated. However, the potentially beneficial characteristics, such as relatively high levels of intercellular airspace, need to be investigated in other species in relation to high CO_2 and low O_2 and known landfill performance. If there is a general association between such species characteristics and landfill performance this will considerably facilitate plant species selection for landfill revegetation. This is discussed further in the next section.

6.3 FUTURE LANDFILL MANAGEMENT AND RESEARCH IMPLICATIONS

Successful revegetation of contaminated or difficult sites is often through the use of plant species that have known tolerances to the problematic environmental factors, especially when used in conjunction with ameliorative procedures that are focused on these environmental factors (Bradshaw, 1984).

Other than this study, little research in South Africa has been done on the tolerance of indigenous species to landfill environmental conditions. The screening of indigenous species suitable for landfill revegetation, by field experiment, has been carried out in Europe and America, however the task is somewhat daunting on the African continent. For example KwaZulu-Natal alone has over 750 indigenous tree species, which is over ten times as many tree species as are native to the whole of Europe (Pooley, 1994). The biodiversity is high and our ecological knowledge about individual tree species is very limited. Thus, it is certainly not possible to investigate all of the species through field trials of relatively long duration and the random selection of tree species has high costs relative

to success or benefits. Therefore, the knowledge we have about the environmental variables that are a problematic for plant growth and the characteristics of species that have performed well on landfills need to be used to increase the efficiency and success of species selection for landfill revegetation. Although this study provides information about the suitability of 10 indigenous species for landfill revegetation, it is the knowledge about the characteristics of these species and the key landfill conditions that determine species success, which are the tools that will be useful for landfill practitioners. They can assist in further species selection and amelioration of landfill conditions to ensure greater success of landfill revegetation.

Landfill gas infiltration into the root zone has been identified as a key variable responsible for poor plant survival and growth. More specifically species that can grow under elevated soil CO_2 conditions need to be identified. Especially those that can tolerate the enhanced negative effects created by low soil O_2, low soil moisture, and high soil conductivity. Although all species appear to prefer to avoid the elevated soil CO_2 and low O_2 conditions through shallower rooting, the selection of species based on their ability to maintain a functional root system when avoidance is not beneficial is likely to yield useful species. This ability may be related to inherent high levels of root and stem porosity and the presence of root aerenchyma tissue, therefore these characteristics could be used as initial selection criteria. It is also apparent that the natural habitat of a species can provide an indication of its potential performance on a landfill. This study indicates that the screening of species that naturally occur in waterlogged habitats will yield a number of useful species for landfill revegetation.

Applying knowledge about the limiting soil variables and other factors causing plant death is important in site preparation for revegetation. The application of topsoil over the normal landfill cover material significantly improved the health and survival of most species in this study. This appeared to be mainly due to the reduced additional effect of high soil conductivity and low soil moisture on the impact of elevated soil CO_2. However, ameliorating high soil CO_2 levels is more problematic. Procedures to reduce landfill gas infiltration into the root zone of plants have been suggested by a number of researchers. These mainly involve barriers or liners below the topsoil layer that divert landfill gas away from the root zone of plants (Gilman *et al* 1985; Spreull & Cullum 1987). In operational sites these measures work similarly to the final landfill cap in separating the surface soils from the underlying waste. This may be a useful technique, however capping material is expensive and for large areas that are only temporarily closed for several years the expense may be restrictive. Therefore, amelioration of the soil CO_2 levels is limited and selection of species tolerant to these conditions appears to be, if possible, the most appropriate solution.

The mechanism by which the concentrations of Mn in the soil increased by six fold in the topsoil placed over the landfill cover material, in this study, needs further research. It has been concluded that the increase is not due to soil contamination from the underlying waste. The results suggest it may be due to changes in soil redox potential due to the low O_2 conditions. However, the relationship between extractable Mn and soil redox potential needs to be researched and the possible impact on plants needs to be determined.

The importance of CO_2 raises questions about the role of methane oxidation in the surface soils and the success of vegetation establishment. Methane oxidation utilises available soil O_2 and results in conversion of relatively harmless methane into CO_2. The global

contribution of methane from landfills has caused concern about the 'greenhouse' effect (Diot *et al* 2000). There is an ever-increasing interest in methane oxidation in landfill cover soils as a natural treatment method for reducing methane emissions into the atmosphere (Visvanathan *et al* 1999). Methane is reported to be 20 times more effective at trapping heat in the atmosphere than carbon dioxide (Haarstad, 1997). Therefore, in order to reduce the 'greenhouse' effect, it may be said that there is a social demand for higher rates of methane oxidation into carbon dioxide, by bacteria in landfill cover materials (Borjesson & Svensson, 1997; De Rome *et al* 1997; Visvanathan *et al* 1999). This demand has resulted in a surge of research into methods of enhancing methane oxidation in landfill cover soils (Boeckx & Van Cleemput 1996; De Visscher *et al* 1999; Willison *et al* 1996). However, methane oxidation increases the levels of carbon dioxide and reduces the levels of oxygen in the soil (De Rome *et al* 1997; Dobson & Moffat, 1994; Haarstad, 1997; Hoeks, 1983). This could make revegetation and stabilisation of landfill sites more difficult. Therefore there is a need for research into the possible implications that enhancing methane oxidation in cover soils may have on revegetation success, as it is clear that the objectives can be in conflict

Research into landfill revegetation allows for a greater understanding of the inter-relationships between the environmental variables resulting in poor vegetation growth, and the mechanisms of species tolerance to these conditions. With this knowledge, management guidelines for the revegetation of operational and complete landfills can be designed, which can help ensure long term successful site rehabilitation, and thus site closure permiting and sustainable land use for future generations.

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Ecological Aspects of Vegetation Establishment on Landfills

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ABSTRACT

A high level of plant mortality on the Bisasar Road landfill, Durban, South Africa initiated an investigation into the primary causes of the mortalities and a search for potentially tolerant plant species. Field studies revealed that volunteer grass growth on cover soils was primarily limited by elevated soil CO_2, with high soil conductivity and low soil moisture possibly compounding the effect. *Cynodon dactylon*, the most abundant coloniser of the site appeared to be relatively sensitive to high soil CO_2, whilst less common species such as *Sporobolis africanus* and *Paspalum paspoloides* appeared to be less sensitive.

Further research focused on the high mortality of trees planted on the landfill providing insight into the important variables limiting survival and the relative differences in performance of 20 tree species. A more rigorous 14-month field experiment was designed and constructed, to assess the performance of 10 of the more promising tree species, the environmental conditions limiting tree growth and the benefit of a deeper layer of better quality topsoil. Some species, such as *Barringtonia racemosa*, performed relatively well in the field experiment, whilst other species such as *Syzygium cordatum*, and *Harpephyllum caffrum* experienced high mortalities and poor growth. The better quality topsoil layer provided little improvement in the performance of the stronger or the weaker species, however significant improvements were recorded for species with relatively intermediate performance. The composition of the soil atmosphere was shown to determine rooting depth. Species that performed better had deeper roots, possibly assisting them in utilising deeper soil moisture reserves. It was concluded that high soil CO_2 and low soil O_2 levels were the key variables responsible for poor tree survival and growth in this field experiment.

A soil fumigation system was designed to provide more control of soil gas concentrations and to experimentally investigate differential species responses and the relative effects of soil CO_2 and O_2 on tree survival and growth. The apparatus fumigated, for a period of 140 days, the rhizosphere of 80 potted 'tolerant' (*Barringtonia racemosa*) and 'non tolerant' (*Harpephyllum caffrum*) trees with 4 treatments consisting of varying combinations of CO_2 and O_2. The difference in performance of *Barringtonia racemosa* and *Harpephyllum caffrum* in the experiment on the landfill was similar to that of the elevated CO_2 low O_2 fumigation treatment, supporting the premise that landfill gas was the key cause for poor performance of plants. Reduced stomatal conductance and resultant limitations on photosynthesis were found to be indicative of species sensitivity. Low O_2 had an additive effect on the impact of elevated CO_2 in *Harpephyllum caffrum* however, even with normal soil O_2 levels, 25% soil CO_2 had negative growth effects on this sensitive species. Maintenance of plant health and better performance of *Barringtonia* was attributed to a high inherent level of tissue porosity and aerenchyma. The research provided a greater understanding of the causes of poor vegetation growth and the possible mechanisms of species tolerance to landfill conditions.

PREFACE

The experimental work described in this thesis was carried out in the School of Life and Environmental Sciences, University of Natal, Durban, from January 1996 to December 2002, under the supervision of Professor J.A. Cooke.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Douglas Trotter

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CHAPTER 1: GENERAL INTRODUCTION

1.1 SOLID WASTE AND LANDFILLS IN SOUTH AFRICA

Waste can be generated from residential, commercial or industrial activity and can consist of unwanted by–products or the remainder of any process, be it a gas, liquid, solid or a combination. It is estimated that South Africa produces between 340 and 480 million tonnes of solid waste annually (CSIR, 1991 from WRC, 1995). More recent estimates of waste volumes suggest that approximately 42 million m^3 of general waste is produced per year across the country (Burger, 2001) The major sources of solid waste are shown in Table 1.1.

Table 1.1: Sources of waste generation in South Africa

Source of waste	Annual Production ($x10^6$ tonnes)
Mining	238.5
Fly Ash	22.2
Agriculture	20.0
Municipal Waste	15.0
Chemical Waste	12.2
Sewage Sludge	12.0
Metallurgical Waste	5.4
Unclassified	4.8

CSIR, 1991 from WRC, 1995

A well-managed sanitary landfill provides an economically and safe means of waste disposal. A sanitary landfill site is a carefully selected, designed and managed waste disposal and containment operation. The waste delivered on a daily basis to the site is spread compacted and covered with soil according to a pre-planned site development programme. Waste deposited in landfills can be broadly classified as general or hazardous waste. General waste includes rubble, garden refuse, domestic waste, commercial waste and general dry industrial waste. Hazardous waste includes any matter, which has toxic

chemicals or long lasting properties, which may have a harmful effect on human health or the environment. The level of site regulation and control determines what types of waste are acceptable for disposal at a site. General waste is disposed of in G classified sites, low hazardous material in H:h classified sites, whilst highly hazardous material can only be disposed in H:H classified landfills which have the highest level of regulation and control.

Of the total waste produced in South Africa an estimated 12 million tonnes is disposed of in sanitary landfills (Jarmain *et al*, 1994) of which South Africa currently has 638 operational sites, 49 officially closed sites and 43 proposed new landfills (Burger, 2001). The odour, noise, dust and visual impact of these operational landfill sites can disturb surrounding communities and trigger more serious concerns about impacts on community health and property value. The use of vegetation with careful landscaping and the construction of berms (an artificial ridge or embankment) can stabilise the completed sections of the site, reduce dust, absorb noise and improve the visual impact of the site (Zeiss & Atwater, 1993). Thus, the successful establishment of trees and grasses on operational landfills can make a vital contribution towards reducing the impact of the site. Successful establishment of vegetation is also essential on completed landfills. Due to the production of flammable gas and site settling as the waste degrades, rehabilitation of closed landfills is usually limited to parks, sports fields and other similar amenity after-uses (Aplet & Conn, 1977; Cooper *et al* 1997; Gilman *et al* 1982; Robinson & Handel, 1995), all of which require successful vegetation establishment. However, the revegetation of landfills throughout the world has met with many difficulties due to the harsh environmental conditions commonly found on landfills (Chan *et al* 1997; Chan *et al* 1996; Chan *et al* 1991; Ettala *et al* 1988; Gilman *et al* 1982; Lassini *et al* 1997; Leone *et al* 1983; Moffat & Houston, 1991; Wong & Yu, 1989; Wong, 1988).

1.2 LANDFILLS AND REHABILITATION

In South Africa the Department of Water Affairs and Forestry, state in section 12, Minimum Requirements for Waste Disposal by Landfill (2nd ed. 1998), that the final condition of the site must be environmentally acceptable and there will be no long-term effects on the surrounding area, water regime and population. It also stipulates that vegetation planted for the purposes of rehabilitation, erosion control or beautification must be maintained to ensure it achieves its purpose. There are no further specifications or guidance given as to revegetation. The regulations do however stipulate the need for incorporation of a 'low permeability' layer or cap in the final cover system of landfills, this is a common requirement throughout the world (Fourie, 2002). The 'low permeability' layer reduces rainfall ingress into the waste, which results in less leachate production, and helps control landfill gas escape into the atmosphere. In South Africa the final cover requirements can vary as considerations of regional climatic and site specific conditions are made. However, a typical cover requirement for a large municipal waste disposal site consists of a 300mm compacted clayey 'low permeability' layer covered with a relatively thin 200mm topsoil layer (Fourie, 2002).

Although there are no guidelines in South Africa stipulating what should be planted on landfills during rehabilitation, grass is the most common forming sport fields or open grassland. However, recently in South Africa there is a demand for rehabilitation to recognise the ecological diversity of a functional ecosystem and assist in the conservation of indigenous fauna and flora (Strachan *et al* 2002). This promotes the use of a broader variety of plant species and the incorporation of shrubs and trees in the rehabilitation plan.

There have been reservations, internationally, about the use of trees in landfill revegetation. Concern about damage to the integrity of the landfill cap by trees has been expressed. In particular: the penetration of the landfill cap by tree roots; evapo-transpiration resulting in shrinkage and cracking of clay cap; and trees experiencing windthrow may disrupt the integrity of the landfill cap. These concerns have previously resulted in the recommendation in the United Kingdom and the United States that trees should not be planted on landfills that have a 'low permeability' layer (Dobson & Moffat, 1995).

However, no evidence, direct or indirect, has been found to support these potential problems on which the recommendations were based (Robinson & Handel, 1995). These fears have since been proven to be largely a misconception due to the lack of knowledge regarding tree root growth characteristics. In fact, evidence suggests that trees show no threat to the integrity of a clay or geotextile covering on landfill sites.(Dobson & Moffat, 1994; Crook, 1992; Robinson & Handel, 1995; Simmons & Coulter, 1997). Some plants are known to produce extremely deep root systems, however this is largely dependent on the particular soil environment (Dobson & Moffat, 1994; Ruark *et al* 1982). The bulk densities found to prevent tree root growth are usually much lower than the recommended bulk density of engineered clay caps (Dobson & Moffat, 1994; Robinson & Handel, 1995). Roots also tend to avoid inhospitable soil zones such as that created by the underlying waste. These findings have resulted in the latest government guidance in the United Kingdom recommending that trees may be planted on all types of landfills (ODP, 2000; Simmons, 1999).

Research indicates that trees can be planted on capped and uncapped sites without compromising the effectiveness of pollution control systems. This allows for a more varied landscape design on all types of landfills and enables sites to blend better with the surroundings and increases the scope of after-uses (Simmons & Coulter, 1997). However, the usually shallow topsoil depth on landfills and the potential for windthrow of older and taller trees has lead to the recommendation that once trees have reached a certain height a system of coppicing should be implemented so as to maintain their stability (Ballardini & Lassini, 1997).

In the South African context the knowledge that trees can safely be used on capped landfills allows landfill rehabilitation plans to incorporate more complex after-use goals without concern for the integrity of the 'low permeability' layer. However, currently there also questions being raised about the necessity for 'low permeability' layer in the rehabilitation plan. Compacted clay capping systems tend to work well in temperate climates that have an excess of precipitation over evaporation because the clay layer does not dry and maintains its flexibility. However, such systems do not perform as well in semi-arid environments, which most of South Africa is classified, as the clay cap dries and cracks becoming permeable. Therefore, it has been suggested that a cover that stores moisture during particularly wet periods and releases moisture via evaporation and evapo-transpiration during subsequent dry periods is a better option. Such an alternative landfill cover would consist, in its simplest form, of a single layer of silty or sandy soil with negligible amounts of clay. The soil used in this cover would resist linear shrinkage thus maintaining flexibility and not cracking during dry periods. The cover would not be considered a barrier but more a regulator of landfill emissions, as it would control moisture into the landfill and would be designed to promote methane oxidation, thus reducing

landfill gas emission into the atmosphere. Although this explanation of an alternative landfill cover is over simplified, a more detailed explanation and evidence supporting this idea as a viable concept are provided by Fourie, (2002). However, if this concept were implemented the greater level of interaction between underlying wastes and the surface soils used for revegetation would be a major consideration.

Similarly, the cover soils used in operational landfills that require stabilisation and aesthetic improvement using vegetation often do not have any 'low permeability' layer separating the topsoil from the underlying waste. Therefore, there is a demand for knowledge about the interactions between the waste and the soil layers used for plant growth and how this can influence successful vegetation establishment. Furthermore, with the operational life span of landfills often exceeding 30 years the demand for plants that can grow successfully on operational sites is ever increasing.

1.3 PLANTS AND THE LANDFILL ENVIRONMENT

The establishment of vegetation on landfills, especially older sites, which have lower standards of pollution control and restoration, frequently results in high plant mortality and sometimes in complete failure. The unsuccessful establishment of vegetation on landfills has been attributed to many factors which include the following: landfill gas; toxic leachate; elevated soil temperature; shallow soil; poor soil quality; poor soil structure; waterlogging; drought; damage by animals; air pollution; and vandalism (Barry, 1987; Dobson & Moffat, 1994; Graber, 1999).

When refuse is first deposited into a new landfill it still contains oxygen, which results in aerobic decomposition. This primarily produces carbon dioxide and water. Within six months all oxygen within the waste is usually used up and decomposition continues in an anaerobic manner (Flower, *et al* 1981). With very little oxygen within the soil, facultative and obligate anaerobic bacterial populations proliferate. These organisms break down or use various organic and inorganic compounds so as to provide their metabolic energy. However, instead of using oxygen as an electron acceptor they utilise inorganic (anaerobic respiration) or organic (fermentation) substrates as the terminal electron acceptors (Bogner, 1992; Gambrell and Patrick, 1978). The result is a different end products of organic decomposition, by comparison to aerobic environments, such as methane, hydrogen, ammonia, amines, mercaptans, butyric acid and hydrogen sulphide, many of which can lead to poor plant growth and survival (Dobson & Moffat 1994; Gambrell and Patrick, 1978; Leone *et al* 1977). Decomposition usually remains in the anaerobic phase because waste compaction and soil cover limit oxygen diffusion to the immediate surface layers (Flower, *et al* 1981).

A typical landfill gas composition consists of 64% methane (CH_4), 34% carbon dioxide (CO_2) and trace concentrations of a wide range of organic gases. These gases escape through the landfill substrates along the paths of least resistance (Christophersen *et al* 2001; Dobson & Moffat, 1994; Flower, *et al* 1981). The composition by volume of a typical landfill gas is given in Table 1.2.

Table 1.2: Typical landfill gas composition

Component	Typical value (% volume)	Max. value measured (% volume)
Methane	63.8	88.0
Carbon dioxide	33.6	89.0
Oxygen	0.16	20.9
Nitrogen	2.4	87.0
Hydrogen	0.05	21.1
Carbon monoxide	0.001	0.0
Ethane	0.005	0.0139
Ethene (ethylene)	0.018	-
Acetaldehyde	0.005	-
Propane	0.002	0.0171
Butane	0.003	0.023
Helium	0.00005	-
Higher alkanes	<0.05	0.07
Unsaturated hydrocarbons	0.009	0.048
Halogenated hydrocarbons	0.00002	0.032
Hydrogen sulphide	0.00002	35.0
Organosulphur compounds	0.00001	0.028
Alcohols	0.00001	0.127
Others	0.00005	0.023

From Waste Management Paper No. 27 (DoE, 1991b) from Dobson & Moffat, 1994

A survey of 65 sanitary landfill sites in the United States revealed that among all the reported environmental factors potentially limiting vegetation establishment on landfills, high levels of landfill gas in the soil was the main cause (Leone and Flower, 1982). This is not a unique finding and a correlation between the level of landfill gas in the soil and poor plant performance has also been noted in numerous other studies (Bradshaw & Chadwick, 1980; Chan *et al*, 1991; Flower *et al*, 1981; Flower *et al*, 1977; Spreul & Cullum, 1987). The harmful effects of landfill gas are usually attributed to displacement of oxygen and resultant anaerobic conditions within the root zone and not the toxic effects of methane or trace components of landfill gas (Barry, 1987; Cole, *et al* 1978; Dobson & Moffat, 1994; Flower *et al*, 1981). However, carbon dioxide (CO_2) which makes up a large component

of landfill gas is also widely accepted to be a problem for plant growth and survival when above certain concentrations (Arthur, *et al* 1981; Barry, 1987; Chan *et al,* 1991; Dobson & Moffat, 1994; Flower *et al,* 1981). There is not an extensive literature on the impact of elevated soil CO_2 on plants however, some of the available ideas will be reviewed below and then the more extensive knowledge base on plants and low soil O_2 will be discussed.

Most soils contain methanotrophic bacteria capable of oxidising methane in the presence of oxygen into CO_2 and water as given in the following equation:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

Thus, not only is CO_2 a large component of the original landfill gas, a large portion of the methane component can also be converted into CO_2 within landfill cover soils. This can result in further increases in CO_2 levels and further depletion of soil O_2 (De Rome *et al,* 1997; Dobson & Moffat, 1994; Hoeks, 1983). Due to methane oxidation the concentration of CO_2 in landfill gas tends to increase as the mixture of gases gets closer to the soil surface and more oxygen is available (Haarstad, 1997). However, the depth of oxidation can vary with soil structure and is greatest where the diffusion of oxygen from the atmosphere and methane overlap (Kightley *et al* 1995). This is usually within the top 300mm of soil, thus resulting in the depletion of soil O_2 and an increase in CO_2 in the root zone for most plants (Dobson & Moffat, 1994). The methanotrophic bacteria responsible for methane oxidation can also produce intermediate products such as methanol, formaldehyde and formic acid (De Rome *et al* 1997; Brown *et al* 1964). These

intermediate products and high levels of carbon dioxide may exhibit direct toxicity to plants.

The CO_2 concentrations within the soil gas phase of normal soils is between 1-5% (Geisler, 1963; Gendebien *et al* 1992; Santruckova & Simek, 1997). However, under landfill conditions root zone CO_2 levels are commonly found in excess of 15% by volume of the soil atmosphere (Chan *et al* 1991; Gilman *et al* 1982; Leone *et al* 1977; Wong *et al* 1992). Concentrations of CO_2 as high as 43% have been measured at a 30cm depth on landfill sites and concentrations as high as 75% by volume could theoretically occur (Arthur *et al* 1981). A large variation in species tolerance to CO_2 levels has been reported (Arthur, *et al* 1981, Gendebien *et al*, 1992; Leone *et al* 1977), and the lower the oxygen levels in conjunction with high CO_2 levels, the greater the degree of toxicity (Ruark *et al* 1982). Generally CO_2 concentrations in excess 6.5% in the rhizosphere has been found to inhibit root growth and result in poor health of number of plant species (Conlin & Van den Driessche, 2000; Chang and Loomis, 1945; Nobel, 1990). Thus, soil CO_2 levels in landfill soils could have a marked effect on plant growth and survival. The mechanism by which elevated CO_2 in the rhizosphere effects plants is not entirely clear. However, as for other components of landfill gas, CO_2 contributes to the displacement of oxygen and the development of anaerobic soil conditions (Flower *et al* 1981, Gendebien *et al*, 1992). It has also been suggested that the effects of high soil CO_2 may be related to the formation of carbonic acid and the acidification of soil water caused by the dissolution of CO_2 which is highly soluble in water (Santruckova & Simek, 1997). The resultant lowering of soil solution pH and possibly changes to the internal pH of cells has been suggested as one of

the possible factors contributing to CO_2 toxicity (Flower *et al* 1981; Santruckova & Simek, 1997).

There has been much study on the mechanisms by which low soil O_2 conditions cause plant stress. A number of key possible causes of root cell damage have been identified and these include insufficient energy generation to sustain cell integrity; cell poisoning by ethanol formed by alcoholic fermentation and cytoplasmic acidosis caused by the products of anaerobic respiration (Vartapetian and Jackson, 1997). It is important to note that when soil O_2 levels are low, anaerobic respiration is likely to occur resulting in a sharp decline in energy availability (Mathews and van Holde, 1991). The reduction in available energy can also reduce the active uptake of mineral nutrients (Kozlowski, 1986; Veen, 1987). Therefore, low oxygen conditions in the soil can result in potassium, nitrogen, phosphorus, calcium and magnesium deficiencies in plants (Flower *et al* 1981, Leone *et al* 1977, Taiz & Zeiger, 1998).

There are also a number of indirect effects by which low soil O_2 conditions can effect plant survival and growth. Under anaerobic soil conditions the release of organic acids by micro-organisms and the accumulation of carbonic acid from respiration and fermentation often results in soil acidification (Flower *et al*, 1981, Larcher, 1980). Low soil pH and redox potentials often accompany the anaerobic conditions. The resultant reducing conditions can lead to increased metal solubility, such as for iron, manganese, aluminium, copper and zinc. The reduced metals can become more available to plants in higher (toxic) concentrations (Crawford, 1989; Leone & Flower 1982). Interestingly, this increased

availability of metals has the potential to result in phytotoxicty however no effects on landfills other than enhancing the trace metal nutrient status of cover soils has been reported (Leone & Flower, 1982).

Microbial activity under low soil O_2 conditions can also change some of the characteristics of the soil, particularly reducing the organic carbon to nitrogen ratio (Flower *et al*, 1981). Nitrogen deficiencies limiting plant growth are common in anaerobic systems because physical, chemical and biological processes under these conditions favour denitrification and low nitrate assimilation. Denitrification is the reduction of nitrate and /or nitrite nitrogen to volatile gases, mainly nitrous oxide and molecular nitrogen, that may escape into the atmosphere (Gambrell and Patrick, 1978).

The poor air movement in the soil atmosphere, commonly found in anaerobic soils, may cause ethylene, a natural plant hormone, produced by the plant, to accumulate in the root tissue and surrounding soil (Jackson, 1985). However, the decomposition of waste in landfills under low oxygen conditions also produces ethylene that can infiltrate the root zone of plants on landfills (Zacharias, 1995). High levels of ethylene can inhibit plant growth (Pezeshki, *et al* 1993; Seliskar, 1988), cause leaf chlorosis (Gepstein and Thimann, 1981; Jackson, *et al* 1987) and cause plant death (Jackson, 1985). Ethylene typically occurs in concentrations of 180ppm (v/v) in landfill gas (Spruell & Cullum, 1987, Dobson & Moffat, 1994) (Table 1.2). However, it is responsible for a greater than 50% reduction in plant growth and often death at concentrations less than 10ppm (Dobson & Moffat, 1994, Smith and Restall, 1971; Spreull & Cullum, 1987). However, Tosh *et al* (1994) found the

threshold concentration for silver birch (*Betula pendula*) seedlings to be 80 ppm, suggesting that there maybe considerable variation in species tolerance. Nevertheless, ethylene may be an important component of landfill gas in determining plant response and vegetation establishment on landfills.

The concentrations of landfill gases in the root zone can be reduced by active extraction or passive venting of gases, from the decomposing waste, which can be burnt off as a flare or used as a fuel (Flower, *et al* 1981; Leone, *et al* 1977). Another alternative is the establishment of gas barriers, using a compacted clay layer or geotextile, preventing landfill gas infiltrating the root zone (Flower *et al* 1981). These procedures can alleviate the problems associated with resultant poor carbon dioxide, oxygen and possibly ethylene levels in the soil. However, they are expensive and not an option for operational sites where revegetation maybe temporary. It is also difficult to install gas extraction or barrier systems in old closed landfill sites that were designed before landfill gas control measures were considered a necessity. Therefore the use of plant species tolerant to the effects of landfill gas infiltration into the soil are the best option for attaining successful revegetation.

In terms of finding species with potential tolerance to these effects, it has been noted that there are many similarities between the anaerobic conditions caused by landfill gas and that of soil waterlogging (Barry, 1987, Chan *et al*, 1991). This has commonly lead to the proposal that species adapted to soil flooding are potentially suitable for planting on landfills (Arthur *et al* 1981; Gilman *et al* 1985; Leone *et al* 1977; Zhang *et al* 1995). The most widespread anatomical feature conferring tolerance to flooded soils is an

interconnected system of gas spaces (aerenchyma) within the root and stem of plants (Jackson, 1994). Aerenchyma results in a lower number of energy-demanding cortical cells in the roots, thus lowering the demand for oxygen (Drew & Fourcy, 1986; Drew & Saker, 1986). It also enhances internal oxygen diffusion and allows oxygen transport from the shoots to the roots (Jackson & Attwood, 1996; Kludze *et al* 1994; Wiedenroth, 1993). Aerenchyma tissue can also result in the oxidation of the rhizosphere, thus alleviating some of the problems associated with low redox potentials caused by anaerobic soils, such as metal toxicity (Blom, 1999; Crawford, 1989). Therefore, species with characteristics conferring tolerance to flooded soils may have attributes that would be beneficial for growth and survival in soils infiltrated with landfill gas.

Apart from landfill gas other interactions between the underlying waste and cover soils, such as heat transfer and leachate contamination can cause changes to the soil that could limit plant survival and growth. The temperature of landfill soils is frequently higher than that of native soils because anaerobic decomposition of waste is exothermic (Flower *et al* 1981, Gilman *et al* 1981, Maurice & Lagerkvist, 1997). High soil temperatures are usually associated with high landfill gas emissions, as warm landfill gas infiltration into the cover soils is usually the key mode of temperature transfer from the waste (Chan *et al* 1991). Elevated temperatures of between 30-40 °C are often measured within the topsoil of landfill sites (Chan *et al* 1991; Dobson & Moffat, 1994; Moffat & Houston 1991), sometimes the temperature difference can be greater than 30 °C between anaerobic and adjacent aerobic soils (Leone *et al* 1977).

Root growth has been found to decrease significantly within the temperature range of 25-35 °C (Ruark *et al*, 1982). Therefore it is not surprising that elevated soil temperature has been identified as a potential problem for plant growth on landfills (Gilman *et al*, 1982; Moffat & Houston, 1991). Although, the higher soil temperature on landfills can prevent the winter freezing of soil water and extend the growing season of many plants in colder countries (Chan *et al* 1991). In the sub-tropical climate of southern Africa the freezing of soil water is seldom encounted. In such tropical climates the raised soil temperature is likely to present a problem for vegetation growth, especially, if one considers that higher soil temperatures enhance the oxygen demand of the root in a soil low in oxygen (Flower *et al* 1981; Gendebien *et al* 1992). However, the amount of heat transferred from the decomposing waste can be alleviate by greater soil depth, the further plant roots are from the source of heat the closer the soil temperature is to ambient (Moffat & Houston, 1991).

An assessment of the impacts of soil leachate contamination on landfill cover soils is not simple. Leachate is the diverse mixture of dissolved and suspended organic and inorganic materials formed when the products of biodegradation mix with the downward migration of water through a landfill (Cooper *et al* 1997). The composition of leachate changes with time as the biodegradation process proceeds, it will also vary with the disposal of wastes of different composition. An example of leachate composition from a recent and an aged domestic waste disposal landfill is given in Table 1.3. With the onset of anaerobiosis as molecular oxygen is depleted the redox potential falls and increases the concentrations of soluble reduced-state metals, such as iron and manganese (Rees, 1982). These metals precipitate as sulphides, hydroxides and carbonates as the pH rises (Rees, 1982). This

results in a considerable reduction in the concentrations of these metals in leachate as the landfill ages (Table 1.3).

Table 1.3: Typical composition of leachates from recent and aged domestic wastes (all figures in mg l^{-1} except pH)

	Leachates from recent wastes (3 years)	Leachate from aged wastes (10 years)
pH	6.2	7.5
COD (Chemical oxygen demand)	23800	1160
BOD (Biochemical oxygen demand)	11900	260
TOC (Total organic carbon)	8000	465
Fatty acids	5688	5
Ammoniacal-N	790	370
Oxidised-N	3	1
p-Phosphate	0.73	1.4
Chloride	1315	2080
Sodium (Na)	960	1300
Magnesium (Mg)	252	185
Potassium (K)	780	590
Calcium (Ca)	1820	250
Manganese (Mn)	27	2.1
Iron (Fe)	540	23
Nickel (Ni)	0.6	0.1
Copper (Cu)	0.12	0.3
Zinc (Zn)	21.5	0.4
Lead (Pb)	8.4	0.14

Adapted from Christensen *et al* 2001 and Fell, *et al* 1993

When leachate is not properly contained it can contaminate ground water, surface water and surrounding soils (Dobson & Moffat, 1994; Gordon *et al* 1989; Menser *et al* 1979). Sometimes collected leachate is recirculated and put back into the landfill in order to promote natural filtration and the microbial decontamination of the leachate (Menser *et al* 1983; Townsend *et al* 1994). The irrigation of landfills with leachate increases the moisture of the landfill, which can benefit the micro-organisms responsible for waste decomposition and stabilisation (Towsend, *et al* 1994) and help with plant moisture requirements (Maurice *et al* 1997). However, irrigation with leachate or when uncontrolled leachate contaminates cover soils it can have a negative effect on vegetation (Menser *et al* 1983; Tong & Wong 1984). This has been attributed to excessive salinity created by the leachate, thus causing osmotic and ionic stress in plants (Ettala, 1988, Cureton *et al*, 1991, Menser *et al*, 1983). Leachate conductivity generally ranges from 0.2 Sm^{-1} to 0.9 Sm^{-1}. An analysis of the effects of leachate indicate that leachate with an electrical conductivity between 0.2-0.4 Sm^{-1} tends to cause slight to moderate tree injury (Bradshaw & Chadwick, 1980). Leachate contamination of cover soils can also result in soil pH changes beyond the normal range (4.5 - 8) suitable for vegetation (McKendry, 1996). High values of particular elements in leachate can also have negative effects on plants. High levels of chloride can result in foliar chloride levels between 2000- 7000 mg kg^{-1} which is within the range of chloride toxicity resulting in symptoms such as leaf discoloration and leaf loss (Menser *et al* 1983; Ettala, 1988). High concentrations of heavy metals in leachate may also result in phytotoxicity. Rainfall and evaporation influence the effects of leachate. An increase in rainfall will result in leachate dilution and lower concentrations, which may be below levels of phytotoxity. However during drier seasons evaporation will result in higher concentrations and the potential for greater negative impacts.

The depth and quality of landfill cover materials is also an important factor determining the success of vegetation establishment on landfills. Completed landfill sites are usually clay capped and covered with a layer of topsoil. Areas of an operational site which have been out of use for any length of time are usually covered with waste soils, layered with topsoil and vegetated so as to aesthetically improve the site. The depth of the topsoil layer can influence the success of revegetation. Shallow soils are prone to waterlogging, desiccation and are also found to restrict the root growth, thus reducing nutrient uptake and anchorage (Dobson & Moffat, 1994, McKendry, 1996). Shallower soils are suitable for grasses and shrubs, which have shallow root systems. For a general vegetation cover 50-100mm soil depth is sufficient (Ettala *et al* 1988). However, when planting trees special consideration of soil depth needs to be made. Trees planted in shallow soils often die or have poor health, and due to insufficient anchorage, are susceptible to windthrow (Dobson & Moffat 1994). For the development of trees a minimum soil depth of 1m is recommended (Dobson & Moffat 1994; Gilman *et al* 1985). A soil depth greater than 2m would be considered unnecessary as the majority of trees roots do not penetrate more than 1.5m (Dobson & Moffat, 1994). A survey conducted by Ballardini and Lassini (1997) on 13 tree species growing on a landfill indicated that if the site was not sealed (clay capped) a topsoil layer of 1.5m could also be regarded as excessive, as landfill gas infiltration limited rooting depths.

Cover soils are not always easily available and are often expensive either due to actual cost, transport costs or both. The expense and availability of cover soils usually results in the utilisation of whatever soil is available at the time and the minimum possible amount is usually used. These soils frequently have poor structure and low nutrient content (Flower

et al 1981). Sometimes before a site becomes operational the original topsoil layer is removed and stored for the later restoration of the landfill. Unless this is done correctly i.e. stored in different horizons, handled only when dry, and not stored for excessive amounts of time, the quality of the soil rapidly deteriorates (McKendry, 1996; Williamson *et al* 1982). The nutrient content may be considerably reduced and the physical structure of good topsoil destroyed by poor handling practices (Williamson *et al* 1982; Cole *et al* 1978).

The most readily available soils are usually of poor quality, comprising a mixture of building rubble, stones, sands, clay and general unwanted soil material. The wastes that have been deposited are covered with soils so as to reduce smells, rodents and waste being blown off site. In order to get the maximum amount of waste into a landfill, specialised vehicles that are used to move the waste into position are designed also to compact the ground at the same time. The action of these vehicles and the general heavy vehicle traffic found on landfill sites results in a very high compaction of waste and cover soils.

The poor quality soil and the high degree of compaction results in poor soil structure for vegetation growth (Heilman, 1981; Insley & Carnell, 1982). A good soil should have sufficient coarse pores to facilitate soil aeration, downward drainage of excess water, and growth of plant roots. However, it is also essential to have sufficient fine pores to retain water. These properties of a soil are very vulnerable and can be destroyed by compaction during soil storage and mechanised earth moving, especially when wet. The living components of the soil, such as worms, fungi etc. which are important in developing and

maintaining structure and fertility tend to be the first to be affected in earth moving processes (Greacen & Sands, 1980). Thus soil compaction is a major consideration in successful tree growth on landfill sites because it is responsible for reduced pore space, aeration, water holding capacity and root penetration (Flower *et al* 1981; Greacen & Sands, 1980; Liang *et al* 1999). Bulk density is a measure of weight per unit volume oven dried soil and refers to the relationship between soil density and pore space. Plant root growth is found to decrease in compacted soils, with root growth decreasing in a linear manner in relation to bulk density (Heilman, 1981). Plant roots will rarely penetrate light textured soils with a bulk density greater than 1.7- 1.8 gcm $^{-3}$ or a heavy textured soil with a bulk density greater than 1.5- 1.6 gcm^{-3} (Dobson & Moffat, 1994). Guideline standards for the main soil variables, which are required for the establishment of trees on a landfill site are given in Table 1.4.

Table 1.4: Minimum standards for soil forming materials acceptable for woodland establishment on landfill sites.

Component	Minimum standard
Bulk density	<1.5 gcm $^{-3}$ to at least 50cm deep
Stoniness	<40% by volume with few stones greater than 100mm
pH	4.0-8.0
Electrical conductivity	< 0.2 Sm^{-1} (1:1 volume soil: water suspension)

Adapted from Moffat and Bending, 1992

The moisture of landfill soils is largely influenced by the degree of compaction. Compaction leads to a higher degree of run-off and less infiltration (Flower *et al* 1981; Greacen & Sands, 1980). However, the soil moisture of a landfill is generally lower than that of the same soil not on a landfill. This is attributed, at least in part, to the reduced capillary rise of water through the refuse and into the cover soils during dry periods. The refuse lacks the capillarity capacity needed for water movement found in normal soils. These periods of reduced moisture in the cover soils of landfill sites has been identified as a potential problem for some plant species in some situations (Gendebien *et al* 1992). The poor soil structure of landfill soil not only results in dry conditions but can also result in poor drainage and waterlogging. Waterlogging often occurs where there are large amounts of uncontrolled leachate production, which together with poor soil structure results in waterlogging and the development of anaerobic soil conditions (Dobson & Moffat, 1994).

Apart from soil variables there are other possible site-specific factors that maybe involved in limiting plant growth on landfills. Poor silvicultural practices and tree maintenance often play a large role in the success or failure of trees planted on landfill sites. Planting of trees by unqualified or poorly trained personnel, inappropriate planting stocks and ineffective weed control is often found to be the causes of failure in revegetation projects (Dobson & Moffat, 1994; Insley, 1980). Further disturbance may be caused by animals such as rats, moles and caterpillars which can be responsible for damage to plants.

The damage to vegetation after establishment is often a problem, especially if areas of the site are still in operation. The movement of heavy vehicles can result in accidental damage

to plants (Ettala, 1988). Operational landfill sites often require unplanned structural changes so as to control rainwater runoff or gas migration. Such alterations may disturb areas, which were vegetated. Vegetation to improve the poor aesthetics of an operational site will inevitably experience some kind of disturbance. The dust, produced by wind and movement of heavy vehicles, can cause the stomatal pores of plants to become blocked, thus, reducing transpiration and gaseous exchange. Large amounts of wind blown rubbish such as plastic bags can get caught in tree branches. For younger trees plastic and paper caught in their branches can result in the branches breaking and increase the possibility of windthrow. Landfill sites are often positioned near industrial areas where the emissions of phytotoxic gases such as sulphur dioxides or fluorides may be problematic. These emissions are known to effect the health of vegetation and could add to the stresses already presented by landfill conditions.

It is clear that a landfill environment has numerous factors that can limit the success of vegetation establishment. However, as with most activities that result in land degradation, the key to rehabilitation is through the use of suitable plant species. There is international literature which discusses plant species selection for landfills, however, no studies on indigenous South African species have been published. Research on suitable species for landfill revegetation appears to have been particularly productive in Hong Kong, United Kingdom, U.S.A and to a lesser extent in Finland (Table 1.5). Variability in species performance on landfills has been apparent to all researchers, with particular species having a greater tolerance to landfill conditions than others (Table 1.5). Even though the reasons for poor vegetation growth on landfills are relatively universal species tolerance to landfill conditions will be influenced by climatic differences, thus tolerant species selected

in anyone country may not be suitable for another country. The scope and need for further research, on a greater number of indigenous species from a wider range of geographic areas becomes apparent when one considers that landfilling is the predominant form of waste disposal in the world.

Table 1.5: A survey of plant species and their performance under landfill environmental conditions.

Species	Reported performance	Country	Reference
Abies alba	Tolerant to oxygen deficient soil, therefore may tolerate anaerobic landfill conditions.	United Kingdom.	Dobson & Moffat. 1994.
Abies spp	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Acacia confusa.	One of the most abundant tree species found in a survey of 13 landfills.	Hong Kong.	Chan *et al*, 1996.
	Suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Acer rubrum	Ranked 10th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Aesculus hippocastanum	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Ailanthus altissima	A predominant species naturally colonising 4 landfills in New York.	U.S.A.	Robinson *et al* 1992.
Albizzia lebbek	Suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Alnus glutinosa	Tolerant to landfill soils if aerobic for less than 0.5m.	United Kingdom.	Crook, 1992.
	Considered the most tolerant species when compared to *Prunus avium, Betula pendula, Fraxinus excelsior* and *Quercus robur*	United Kingdom	Mackay & Richardson. 1996
Alnus incana	Tolerant to landfill soils if aerobic for less than 0.5m.	United Kingdom.	Crook, 1992.
Aporusa chinensis	Not suitable for landfill revegetation.	Hong Kong.	Chan *et al*, 1991.
Betula pendula	Tolerant to landfill soils if aerobic for less than 0.5m	[illegible]	[illegible]
Salix aquatica	One of the most productive species growing on six landfills.	Southern Finland.	Ettala. 1988.
	Growth not influenced by high levels of CO_2 in landfill soils in three landfills.	Finland	Maurice *et al* 1997
Salix babylonica	Ranked 18th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Salix caprea	Non survived in 2 year experiment, even with a 0.5m clay or compost layer over the landfill cover material.	United Kingdom.	Moffat & Houston. 1991.
Salix spp.	Tolerant to landfill soils if aerobic for at least 0.5m.	United Kingdom.	Crook. 1992.
Salix viminalis	Damaged by leachate irrigation in a survey of six landfills.	Southern Finland.	Ettala. 1988.
	Growth not influenced by high levels of CO_2 in landfill soils in three landfills.	Finland	Maurice *et al* 1997
Taxus cuspitata	Ranked 2nd most tolerant to landfill conditions of the 19 species screened	U.S.A.	Flower *et al* 1981
Tilia americana	Ranked 8th most tolerant to landfill conditions of the 19 species screened	U.S.A	Flower *et al* 1981
Tilia spp.	Tolerant to landfill conditions if the soil is aerobic for at least 1m.	United Kingdom.	Crook. 1992.
Tristania conferta	Suitable for landfill revegetation.	Hong Kong	Chan *et al* 1991

1.4 AIMS AND OBJECTIVES

1.4.1 Research aims

The nature of landfill environmental conditions makes the successful establishment of vegetation on operational and complete sites difficult. The key focus of this research was on the revegetation problems associated with an uncapped operational site.

The specific aims of this investigation were to identify and quantify the key environmental factors limiting vegetation establishment on the Bisasar Road Landfill, and to assess the relative plant performance of some indigenous tree and grass species. Tree performance was experimentally investigated further both in the field and using a soil gas fumigation system. General physiological attributes were sought which could improve species selection for landfill revegetation.

1.4.2 Thesis structure

An investigation into the micro-distribution of grass species naturally colonising the Bisasar Road Landfill was conducted to assess plant species performance and possible limiting variables (Chapter 2). An investigation into an unsuccessful revegetation attempt, using indigenous tree species, on a stability berm at the Bisasar Road Landfill was conducted, and is described in Chapter 3. This provided preliminary information about the relative tolerance of a number of indigenous tree species and the key variables responsible for poor tree survival. Based on this work, a field experiment was designed in order to make a more detailed assessment of the suitability of 10 indigenous tree species for landfill revegetation. The field experiment also assessed the usefulness of a topsoil layer for improving the survival of trees and provided a comparison with direct planting in the

normal cover soil. This experiment provided an evaluation of some of the variables which limited tree growth and survival (Chapter 4).

In the field investigations and experiment the heterogeneity and dynamic nature of the landfill environment and the high mortality of less 'tolerant' species often made it difficult to explain differential species performance and establish the role of soil CO_2 and O_2 in determining plant health on the landfill. To provide an experimental approach, a soil gas fumigation system was designed and constructed. Using two tree species with different performance from the field experiment, the fumigation system was used to test the hypothesis that differential species performance on the landfill was due to elevated soil CO_2 and low O_2 (Chapter 5). The fumigation experiment aimed to evaluate the relative importance of high soil CO_2 and low O_2 concentrations in determining plant performance as well as the potential for antagonistic, additive or synergistic effects between these two variables.

A discussion of the main conclusions is given in each of the four separate result chapters. A final overall discussion of the results is given in Chapter 6, which considers: key limiting factors for vegetation establishment; plant species response and selection; and the objectives for further research.

1.5 SITE DESCRIPTION: THE BISASAR ROAD LANDFILL

The Bisasar Road Landfill site is situated in the Springfield area of Durban, South Africa. The 21 million cubic meter capacity site first started operation in 1980 and serves the waste disposal need of the city of Durban. The site is bounded to the north by the flood plains of the Umgeni River on which are sited the Clare Estates School and the Solid

Waste, Health and Electricity Departments of the City of Durban. Residential areas along Kennedy, Clare, Burnwood and Dhulam Roads bound the site to the east, south and west. In the south-eastern corner lies the City of Durban Nursery (Figure 1.1). The landfill is located within a north facing, steep sided valley with its floor situated approximately 12m above sea level on the Umgeni river flood plain and the top of the valley situated to the south at approximately 110m above sea level (Figure 2.1). The landfill does not have a clay or geotextile liner.

The underlying geology of the area consists of the Pietermaritzburg Formation of the Ecca Group in the Karoo Sequence. The Pietermaritzburg Formation is extensively intruded by Dolerite sills and dykes of the Jurassic Age and is comprised of predominantly bedded, dark grey to black, carbonaceous shales and micaceous siltstones with occasional bands of thin sandstone. The dolerite intrusions are usually extensively weathered to a yellow orange and reddish brown silty clay. A major Dolerite sill occurs within the eastern side of the valley in which the site is located. Several geological faults are found approximately 500m to the east and the west of the site (Loudon and Partners, 1993).

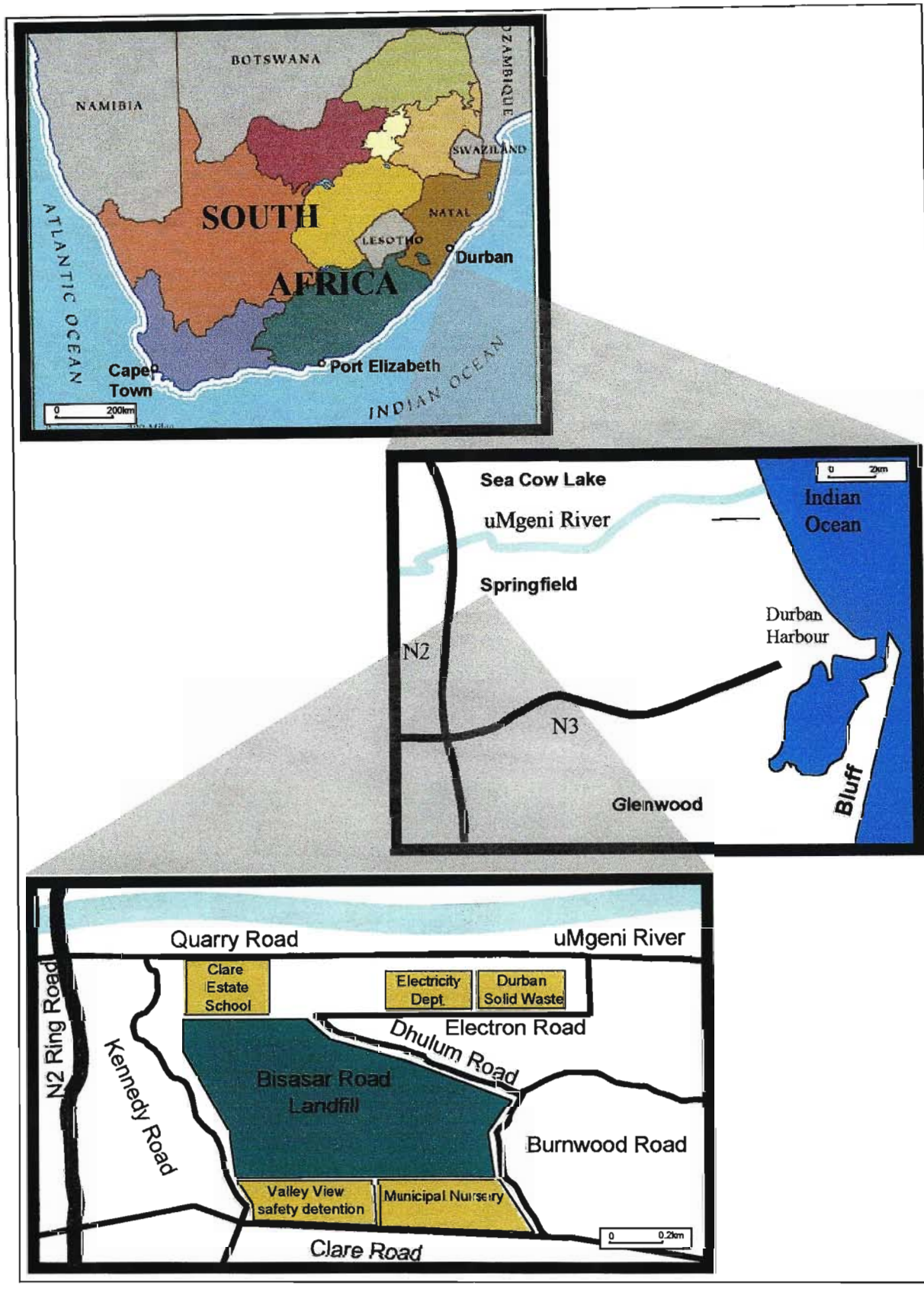


Figure 1.1: Location of Bisasar Road Landfill in Durban, South Africa

The Bisasar Road landfill site is used for the disposal of domestic and general industrial waste. The site is located in the centre of the municipal area, thus minimising the cost of transporting waste for the city, private individuals, contractors and other local authorities. The landfill site serves mainly the Greater Durban Metropolitan Area disposing of approximately 48862 tons of mixed waste on a monthly basis. A break down of the types of waste disposed of can be seen in Table 1.6.

The landfill operation is structured in a series of terraces of waste, which are compacted and covered with waste soils and rubble at the end of each day. These terraces are worked forward until they reach a main stability berm at the base of each main terrace. Each phase of development will have a main containment or stability berm. This berm is usually built with an initial lift of 5m and thereafter lifts of 2m with each set of terraces until the designed filling level of the site is reached. The berm is constructed with non-compactable material such as metal, rock, builders rubble or reinforced concrete. The final outer slope of the berm is then top soiled and vegetated.

Table 1.6: Receipt of waste (average per month, in tons) at Bisasar Road landfill site (Adapted from Lombard & Associates,1994)

Waste types	Monthly Average (tons)
Domestic	13861
Trade	12783
Garden	9143
Cover	11821
Street sweeping	1135
Vehicles	16
Fish	41
Fresh produce	61
Other	1
Total	48862

Annual rainfall figures from the meteorological station at Durban International Airport, 20km south of Bisasar Road landfill, are shown in Table 1.7. The minimum and maximum average summer and winter temperatures are 19 - 26°C and 12 - 23 °C respectively (Michellin, 1990).

At the time of the preliminary investigation (1996) the northern side of the site, directly behind the main stability berm, was not in use, however, the installation of gas reclamation wells was planned for this area (Figure 1.2). The project involved the sinking of 24 wells for the extraction of methane from the underlying decomposing waste, to be used on a commercial scale. Waste disposal was continuing mainly on the southern section of the site which receives approximately 48900 tons of waste per month (Table 1.6).

There has not been any establishment of indigenous woody vegetation on the Bisasar Road Landfill. On the site, and the surrounding disturbed areas alien, invader species such as *Ricinus communis* (castor oil bush), *Solanum mauritianum* (bugweed), *Melia azedarach* (syringa) and other exotics are the main species that occur. The south eastern and north western corners of the site remain permanently wet due to a natural spring and species such as *Phragmites* spp (common reed), and *Bambusa* spp (Bamboo), *Typha* spp (bulrush) are found. The grass cover on the landfill is mainly *Cynodon dactylon,* interspersed with numerous young *Melia azadarach* which have established themselves in areas which have not been utilised for waste disposal for any length of time.

Table 1.7: Average rainfall figures for the year as recorded by the meteorological station at Durban International Airport (1950-1995).

Month	Average Rainfall (mm)	Max. Rainfall (mm)
January	135	310 in 1984
February	126	361 in 1986
March	127	397 in 1976
April	86	283 in 1957
May	60	227 in 1971
June	27	139 in 1961
July	34	147 in 1963
August	59	252 in 1981
September	77	402 in 1987
October	103	251 in 1964
November	112	246 in 1989
December	104	331 in 1958
Summer (Oct. - Mar.)	707	
Winter (Apr. – Sep.)	343	
Total	1050	

CHAPTER 2: THE MICRODISTRIBUTION OF GRASSES FROM VOLUNTEER COLONISATION

2.1 INTRODUCTION

The establishment of vegetation on recently covered landfills is very important for the stabilisation of soils and prevention of erosion (Gilman *et al* 1985). However, many landfills experience stunted vegetation growth and poor cover including bare patches where plants do not grow, thus not achieving either the stabilisation of cover material or the improved amenity value intended (Lan & Wong, 1994; Davis & Coppeard, 1989; Wong, 1988). Poor plant growth on landfills has been attributed to high concentrations of carbon dioxide and methane, low amounts of oxygen, poor soil structure and low soil nutrient availability, all factors that commonly occur in landfill soils (Gilman *et al* 1985).

Grasses tend to survive better than other plant types such as trees and shrubs on landfills, especially where there are high soil concentrations of carbon dioxide and methane (Lan & Wong, 1994). The better survival of grasses has been explained by their shallow rooting depths. The roots remain near the surface and thus avoid the higher concentrations of carbon dioxide and methane experienced at depth in the soil of landfills (Lan & Wong, 1994). Erosion by wind and water is effectively controlled by the closed leaf canopy, relatively high basal cover, and fibrous root systems provided by grasses. They also form a useful 'pioneer' community which may facilitate the development of a more complex vegetation structure on landfills (Zacharias, 1995). Grassland forms a key vegetation type in the revegetation of operational sites and the rehabilitation of landfills into after uses such as parks, gardens and golf courses.

The aim of this investigation was to identify the environmental factors limiting grass growth in certain areas of the Bisasar Road landfill, possibly providing insight into ameliorative procedures needed to achieve a more complete ground cover. The identification of species preferences as to microhabitat conditions sought to identify species relatively more tolerant to landfill conditions.

2.2 MATERIALS AND METHODS

2.2.1 Site description

A temporarily complete section of the Bisasar Road Landfill, Springfield, Durban, South Africa, was naturally colonised by a variety of grass species over an approximate eighteen month period. This section of the site was not clay capped, as waste filling was likely to continue in this area during the future years of the landfill life span. The grass was growing in a 500mm waste soil layer which formed the cover over an approximately 30m depth of domestic waste, filled into the valley since 1989, and which formed a large terrace. This vegetation dominated by grasses had a patchy appearance with bare areas where no vegetation had colonised (Figure 2.1).

Figure 2.1: The vegetation dominated by grasses had a patchy appearance with bare areas that no plants had colonised.

2.2.2 Sampling design and field measurements

Within a temporarily complete section of the Bisasar Road landfill (approximately 15000m^2), four conspicuous patches without grass were selected. The four patches were selected outside of the effective range of the area identified for gas reclamation well installation (Dorkin, D. 1996 *pers comm*). This was to ensure that landfill gas, a potential factor causing the patches, was not altered during the investigation. The area of each of the four bare patches was divided into quarters. Within each quarter a random transect radiating out from the centre of the bare patch into the surrounding vegetation was positioned. Three 0.5m by 0.5m quadrats were placed along each transect, one within the bare patch (no grass); another incorporating the first grasses on the border of the patch (border grass); and

the final quadrat positioned within the first well established stand of grass (established grass). Grass and environmental variables were measured within each quadrat.

The different grass species present within each quadrat were identified. The above ground plant material (live standing crop) for all species was collected from each quadrat and oven dried at 105°C until a constant weight. This was used to calculate species biomass and total biomass for each quadrat (Allen, 1989).

A 400mm long, 22mm outer and 15mm inner diameter plastic pipe was used for gas sampling. The end 200mm of the pipe was perforated with sixteen 5mm diameter holes and inserted 300mm below the soil surface in each quadrat. A more complete description of the gas sampling pipe is described in Chapter 3, however, it differs in length by 600mm. Due to the high compaction and large stone content, the hole in the ground into which the gas samplers were inserted had to be drilled with a 38mm masonry bit. Although the drill bit had a greater diameter than the sampling pipe the slight subsidence of the hole wall after drilling resulted in a close fit between the hole and sampling pipe. The gas samplers, once inserted into the hole, were tightly packed into the ground and sealed with airtight caps. They were allowed one week to equilibrate with the soil atmosphere before percentage methane, carbon dioxide and oxygen in air were measured using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. The soil temperature at a 200mm depth for each quadrat was recorded by inserting a digital thermometer (YFE YF-1062) into each gas sampler.

2.2.3 Soil analysis

A soil sample was collected from the surface to a depth of 150mm from each quadrat. The single sample from each quadrat was immediately sealed into a plastic bag and then mixed. Two sub-samples of soil from each quadrat were analysed for percentage moisture content by oven drying at 105°C (Grimshaw, 1989). A sub-sample of soil from each quadrat was sent to the Kwazulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: extractable P; K; Ca; Mg; Zn; Mn; sample density; extractable acidity (titrated NaOH expressed as centimoles of acidity per litre of soil); pH; % organic carbon; % clay (Hunter, 1974). A description of the techniques used for these analyses is provided in Chapter 4 (section 4.2.4).

The remainder of the soil was air dried and passed through a 2mm sieve, separating the soil from the stone. The stone content was then calculated as a percentage weight of the original sieved sample. For conductivity measurements approximately 20g of sieved soil was saturated with de-ionized water and allowed to stand for 24 hours. The high clay content of the soil samples made it difficult to extract any filtrate using a Buchner funnel and filter paper under suction with a vacuum pump, therefore, centrifugation was used instead. The soil water mixture was centrifuged using a Beckman G.P. centrifuge (No. 355953) at 3700rpm (relative centrifugal force = 2127.4) for 30 minutes to extract the supernatant of which the conductivity was measured using a Crison MicroCM 2201 conductivity meter corrected to 25 °C.

Statistical analysis of the data collected was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data was analysed using analysis of variance. If there was a

significant difference ($p<0.05$) in data with more than two samples a Sheffe Multiple Range test was performed to determine which differences were significant ($p<0.05$). The relationship between grass biomass and the environmental conditions measured was also evaluated using a scatter plots and Pearson's Product-moment Correlation Analysis.

2.2.4 Bioassay

A soil sample (approximately 2kg) taken from each quadrat on the landfill was air-dried and sieved with a 2mm sieve. Decomposing waste material below the soil cover of the area investigated would be the main cause of potentially high carbon dioxide, methane and low oxygen in the soil. Therefore, the removal of the soil samples from the site would change these conditions, which could be effecting plant growth, thus, returning the normal gas composition to the soil atmosphere. Further, sieving of the soil removed the stones and altered the original field structure of the soil, thus improving the physical aspects of the soil which might be causing poor grass growth in the field.

The sieved soil samples from each quadrat were placed into 350ml plastic containers (Container Corporation) with holes drilled in the bottom. Stolons from a single *Cynodon dactylon* parent plant were cultivated in seedling trays in a glass house for 3 weeks. The resultant genetically similar plants of similar size were selected and the shoots were trimmed to the same height. Twenty of these plants were randomly selected and oven dried at 105°C so as to provide a figure for the original mean root and shoot weight of the plants to be used in the bioassay. Forty-eight plants were then planted into the plastic containers giving a single plant in each container, which contained soil from a particular quadrat. This gave four replicate plants for each of the three areas (no grass, border grass and established grass) of

each patch, totalling 12 plants for each of the 4 patches and 48 plants/containers in all. So as to provide a control another 6 plants were put into similar containers containing potting soil. The plants were grown for 4 weeks under random block design in a controlled environment chamber (Conviron), provided with 12 hours light at 25°C, 12 hours dark at 18°C and watered once a week ensuring that the soil remained moist.

If the causal environmental conditions in the bare areas on the landfill were resulting in a chronic response in plants, then some short term but permanent attempt at colonisation of the bare areas would be apparent. Therefore, the bare areas would not be totally void of vegetation but would be characterised by stunted and sickly young plants attempting to colonise the area. Considering the areas without grass on the landfill had no vegetation at all it was assumed that the causal environmental conditions was resulting in an acute response in plants and thus no vegetation growth was found. Therefore, a one month period for the bioassay was thought to be sufficient to elicit a detectable response in the grass planted.

After the four week period the plants and soil were carefully removed from the containers and the soil washed from the roots. The plants were then oven dried at 105° C until a constant weight. The dry weight of the roots and shoots of each plant was then measured. The root and shoot weights of the four plants grown in the soil from the each of the four quadrats in each area, namely the no grass area, border grass area and established grass, of each patch were compared using an analysis of variance. The root and shoot mass data from the different areas for the four patches were then pooled together (n=16) and again analysed using an analysis of variance and Sheffe Multiple Range test.

2.3 RESULTS

2.3.1 Field sampling

Twelve different species of grasses were identified in the area of investigation on the Bisasar Road Landfill Site. These were found with different relative abundances and distributions (Table 2.1). *Paspalum paspalodes distichum, Cynodon dactylon, Sporobolus africanus* and *Panicum maximum* were the most common species with the highest frequency in the quadrats with grass and with the highest overall standing crop. *Paspalum paspalodes* was only found in patches 1 and 2, but had a relatively large biomass in the border areas in comparison to the other species (Table 2.1). Similarly, *Sporobolus africanus* had its highest biomass in the border areas of all three of the patches in which it was present. *Panicum maximum* was only found in patches 3 and 4, and had a relatively larger biomass in the established stands of grass, especially in patch 4. *Cynodon dactylon* was the most abundant species in terms of biomass and frequency and was found in all patches. However, the absence or relatively low biomass of *Cynodon dactylon* in border areas in comparison to the well-established stands of grass was apparent. These results showed that *Paspalum paspalodes* and *Sporobolus africanus* were the main species found in the borders of the areas where grass did not grow, whilst *Cynodon dactylon* and *Panicum maximum* were predominantly found in the established stands.

Many of the species were only found in one or two quadrats and had relatively low total biomass making it difficult to make any conclusions about their distribution other than that they were relatively uncommon species. However, it was noted that six of the twelve species were only found in border areas (Table 2.1). These species were *Chloris gayana, Digitaria eriantha, Echinichloa colona, Eragrostis curvula, Paspalum urvillei,* and

Sorghum bicolour. All of the six other species were found in both the established stands and the border areas (Table 2.1). It was clear that the border areas had higher species diversity in comparison to the established stands.

Table 2.1: Mean above ground biomass (dry mass (g) / 0.25m^2) of each grass species for the border area (B) and surrounding established grass (EG) for the four patches investigated on the Bisasar Road Landfill site.[1]

Grass Species	Patch1		Patch 2		Patch 3		Patch 4	
	B	**EG**	**B**	**EG**	**B**	**EG**	**B**	**EG**
Chloris gayana	6.5[1]	0	0	0	0	0	0	0
Cynodon dactylon	0	62.8	0	276.8	4.1	104.6	11.7	55.9
Dactyloctenium	0	2.4	0	0	0	0	0.4	0
Digitaria eriantha	4.6	0	0	0	0	0	0	0
Echinichloa colona	0	0	0	0	0	0	1.0	0
Eragrostis curvula	0	0	0	0	5.8	0	0	0
Melinis repens	0	0	0	0	0.2	0	0	4.1
Panicum maximum	0	0	0	0	1.5	19.3	0	62.2
Paspalum paspalodes	70.3	190.3	47.6	0	0	0	0	0
Paspalum urvillei	0	0	11.9	0	0	0	0	0
Sorghum bicolour	0	0	0	0	0	0	0.9	0
Sporobolus africanus	0	0	8.5	0	44.4	2.2	10.8	10.1

[1]The biomass is the mean of four quadrats in each area for each patch.

The data collected from each of the patches was pooled together in order to increase the sample size and decrease the effect of extreme values. With a larger sample size, significant changes in environmental variables were not as easily masked. Thus, significant differences in environmental variables, which may be a common cause for poor grass growth, could be identified. The pooled data was analysed using an analysis of variance and Sheffe multiple range test (Table 2.2). The environmental variables from all the patches and quadrats were also analysed in relation to the total biomass in each quadrat using a Pearson's product-moment correlation analysis (Table 2.4).

Table 2.2: Soil variables (mean and standard error; n=16) measured in no grass, border grass, and established stands of grass for combined data of the four patches.

Environmental variables	No grass	Border grass	Established grass
Oven dry Biomass (g)	0.0 ±0.0 a [1]	59.8 ±8.5 b	197.7 ±26.1 c
Extractable P (mg kg^{-1})	11.7 ±1.1 a	8.5 ±0.7 ab	8.7 ±0.7 b
Extractable K (mg kg^{-1})	231.7 ±32.2 a	255.9 ±49.7 a	304.2 ±65.7 a
Extractable Ca (mg kg^{-1})	1703.8 ±160.5 a	1222.7 ±100.0 b	1163.8 ±135.1 b
Extractable Mg (mg kg^{-1})	263.4 ±23.9 a	278.1 ±32.7 ab	424.6 ±61.4 b
Ext. Acidity (Cmol kg^{-1})	0.1 ±0.01 a	0.1 ±0.01 a	0.1 ±0.01 a
pH	7.6 ±0.1a	7.7 ±0.1 a	7.8 ±0.06 a
Extractable Zn (mg kg^{-1})	14.5 ±2.3 a	10.0 ±1.2 ab	8.5 ±1 b
Extractable Mn (mg kg^{-1})	45.6 ±9.1 a	35.3 ±6.0 a	52.5 ±9.9 a
Organic carbon (%)	4.4 ±0.2 a	5.1 ±0.4 a	4.2 ±0.3 a
Clay (%)	37.6 ±1.8 a	37.6 ±1.7 a	37.2 ±2.2 a
Moisture (%)	14.2 ±0.6 a	17.6 ±0.9 b	17.7 ±1.1 b
Stone (% weight)	57.0 ±2.6 a	52.6 ±2.2 a	52.5 ±3.7 a
Conductivity ($mScm^{-1}$)	5.2 ±0.4 a	5.9 ±1.0 a	5.2 ± 0.9 a
Methane (%)	17.5 ±4.3 a	15.9 ±4.5 a	8.5 ± 4.0 a
Carbon dioxide (%)	14.5 ±3.2 a	12.4 ±2.9 a	6.8 ±2.3 a
Oxygen (%)	12.4 ±1.6 a	12.4 ±1.6 a	15.5 ±1.6 a
Soil temperature (°C)	25.1 ±0.5 a	24.6 ±0.4 a	23.8 ±0.5 a

[1] The means in the rows across the table followed by different letters are significantly ($p<0.05$) different with a Sheffe Multiple Range test.

The pooled data showed significantly ($p<0.05$) higher concentrations of Zn, P and Ca in the no grass areas in comparison to the established stands with intermediate concentrations within the border area (Table 2.2). However, the Ca concentrations in the border areas were the same as the established grass area. The no grass area was significantly ($p<0.05$) lower in Mg and soil moisture in comparison with the established grass stand. Mg levels were intermediate in the border areas, however, there was no significant ($p>0.05$) difference in soil moisture between the border area and the established stands (Table 2.2).

It is important to note that although there was no significant variation in conductivity, methane, and carbon dioxide concentrations within the patches, the values measured in soil throughout the patches were beyond the normal range expected for healthy soils (Table 2.2).

When the data from each of the individual patches was analysed separately, other significant differences, which were not found with the analysis of the pooled data, were found, these included K, carbon content, Mn, and conductivity (Table 2.3). This suggested that these differences were probably patch specific and were not common for all bare patches.

Table 2.3: Soil variables (mean and standard error; n=4) which had significantly different values measured in no grass, border grass, and established grass in each of the four individual patches.

Soil variables	No grass	Border grass	Established grass
Patch 1			
Extractable Zn (mg kg^{-1})	27.4 ±4.5 a	11.5 ±3.3 b	11.0 ±2.7 b
Extractable K (mg kg^{-1})	284.4 ±33.2 a	278.5 ±41.7 a	478.7 ±82.0 b
Extractable Mn (mg kg^{-1})	33.0 ±3.7 a	59.6 ±13.9 ab	85.6 ±8.1 b
Moisture (%)	12.6 ±0.6 a	21.0 ±0.6 b	21.4 ±1.3 b
Patch 2			
Extractable Mn (mg kg^{-1})	48.2 ±4.6 a	49.3 ±1.4 a	92.2 ±9.1 b
Organic carbon (%)	4.2 ±0.2 a	6.5 ±0.7 b	4.0 ±0.2 a
Patch 3			
Extractable P (mg kg^{-1})	16.4 ±3.1 a	9.2 ±1.8 ab	7.1 ±1.4 b
Patch 4			
Extractable Mg (mg kg^{-1})	150.4 ±17.4 a	216.2 ±33.9 ab	325.2 ±51.5 b
Conductivity (mS/cm)	5.7 ±0.5 a	2.2 ±0.6 b	1.8 ±0.3 b

[1] The means in the rows across the table followed by different letters are significantly different with a Sheffe Multiple Range test.

[2] Significance level, a $p<0.05$; aa $p<0.01$

Using the pooled data the relationship between the total biomass and the variables measured was analysed using a Pearson's Product-moment correlation. The variables which showed a significant correlation ($p<0.05$) were further analysed using a linear regression analysis (Table 2.4).

Table 2.4: Correlation between soil variables measured and the total plant standing crop of the individual quadrats from all of the patches (n=48). The relationship between the variables with significant ($p<0.05$) correlation coefficients was analysed using a linear regression and the R-squared value and the level of significance given.

Environmental variable	Correlation coefficient	Linear regression R-squared value (%)
Phosphate (P)	-0.15	
Potassium (K)	0.37* [1]	13.49**
Calcium (Ca)	-0.47**	21.83**
Magnesium (Mg)	0.62**	37.24**
Exchangeable acidity	-0.22	
pH	0.32*	9.96*
Zinc (Zn)	-0.30*	8.83*
Manganese (Mn)	0.40**	15.24**
Organic carbon	-0.16	
Clay	0.06	
Moisture	0.41**	16.41**
Stone content	-0.21	
Conductivity	0.16	
Methane	-0.23	
Carbon dioxide	-0.30*	9.07*
Oxygen	0.24	
Temperature	-0.46**	20.89**

[1] Significance level * $p<0.05$; ** $p<0.01$

The results for the levels of soil Mg and moisture showed a positive relationship with total biomass (Table 2.4) reinforcing the results indicated by the ANOVA (Table 2.2). There was also a significant ($p<0.05$) positive correlation between K concentrations and grass biomass however, the ANOVA results suggested that K variations were patch specific. Although a

relationship between low soil K and low grass biomass was apparent, the discrepancy between the correlation and ANOVA results made it difficult to determine the importance of soil K in determining grass establishment.

The Zn and Ca concentrations had a significant negative relationship with total biomass (Table 2.4) similar to the ANOVA results. It is interesting to note that the highest r-squared values for the linear regression were for Mg and Ca, suggesting that a relatively large percentage of the variability in biomass was determined by the concentrations of Mg and Ca in the soil. It is also interesting to note that whilst Mg had a positive relationship with grass biomass, Ca had a negative relationship, possibly suggesting that the relationship between soil Ca and Mg could influence the growth of grasses. To asses this the analysis of the ratio Ca/Mg to grass biomass showed a significant ($p<0.01$) negative linear relationship ($R^2 = 0.136$) and a significant ($p<0.01$) negative correlation coefficient (-0.369). However, the correlation coefficient and R^2 value for the relationship between Ca/Mg and grass biomass was less than that of either of the individual nutrients separately (Table 2.4). This indicated that the absolute levels of Ca and Mg in the soil were probably more important, in terms of grass biomass, than the relationship between the two variables.

The results of the correlation analysis for soil organic carbon content, conductivity, methane, oxygen, stone content, % clay, exchangeable acidity and grass biomass further reinforced the ANOVA results showing that there was no relationship between grass biomass and these variables. However, the correlation analysis showed a positive relationship between soil Mn levels and grass biomass yet there were no significant differences between the quadrats with the ANOVA. Similarly the ANOVA indicated that there was a relationship between grass growth and P concentrations yet there was no

significant relationship shown with the correlation analysis. Thus, the variations in Mn and P were difficult to interpret and their relationship with grass growth was unclear. However, similarly to K, the unclear results made it difficult to determine the if the variation in soil Mn and P were likely to explain the lack of grass establishment.

The correlation analysis revealed some relationships between biomass and soil variables that were not identified by the ANOVA. These included soil pH, temperature and carbon dioxide concentration. Soil pH had a significant ($p<0.05$) positive relationship with grass biomass, suggesting that higher grass biomass was found in areas with a higher soil pH. Soil temperature had a significant ($p<0.01$) negative relationship with grass biomass. The higher soil temperature in the no grass areas was probably due to the lack of vegetation, therefore there was greater heating of the soil by the sun. This probably indicates that the soil temperature relationship was more a symptom than a cause of poor grass establishment. It could also have been due to the infiltration of warm landfill gases as indicated by the significant relationship between carbon dioxide and grass biomass. Carbon dioxide had a significant ($p<0.05$) negative relationship with grass biomass, suggesting that higher levels of carbon dioxide were associated with lower grass biomasses. Carbon dioxide concentrations in the soil ranged from 0% to 39% with generally a lower grass biomass at higher gas concentrations (Figure 2.2).

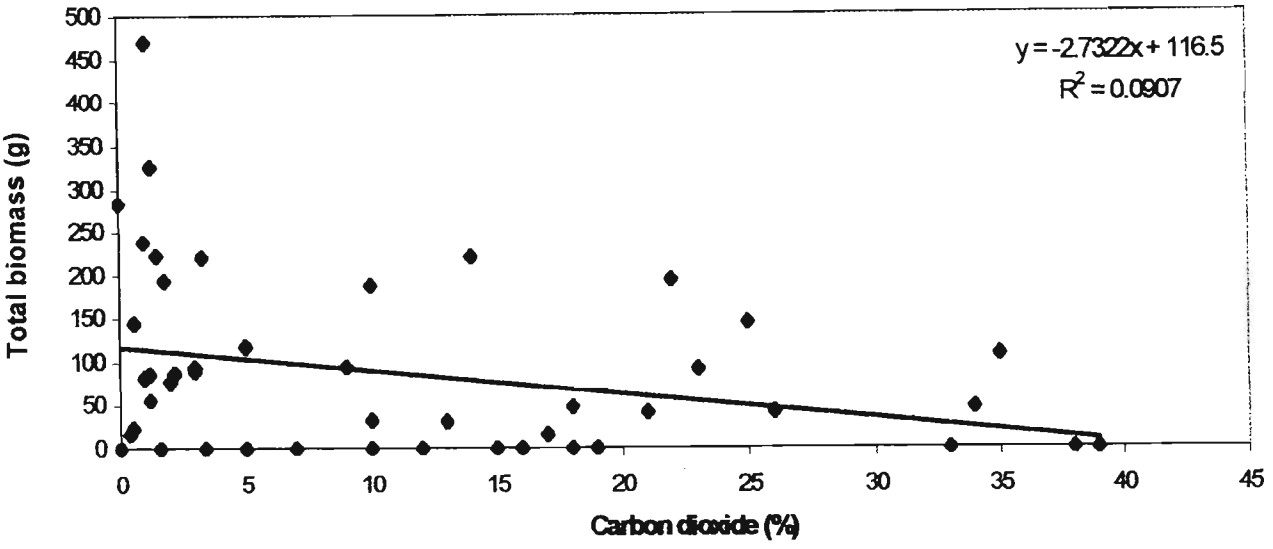


Figure 2.2: Linear regression ($p<0.05$) of the relationship between carbon dioxide and total above ground biomass.

In summary, it appeared that of the variables measured, the higher levels of Mg, moisture and K in landfill cover material were associated with higher grass biomass, whilst high levels of Zn and Ca were associated with low biomass. There was also evidence indicating higher grass biomass was associated with higher pH values and the bare areas were associated with high soil temperature and elevated soil CO_2 concentrations. The data suggested that there may be some relationship between grass growth and soil Mn and P levels however, the results were unclear.

The grass species that were found in more than three quadrats had sufficient data to be subjected to a correlation analysis to determine the relationship between the environmental conditions and the biomass of individual species (Table 2.5). However, with low sample sizes (3 quadrats) the chance of making a type two error is greater than with a higher number of replicates. *Panicum maximum* was only found in four quadrats, therefore, the

probability of correctly rejecting the null hypothesis was low. However, *Cynodon dactylon, Sporobolus africanus* and *Paspalum paspalodes* were found in a larger number of quadrats, namely 14, 8, and 10 respectively, thus increasing the power of the analysis.

Table 2.5: Results of a correlation analysis for the biomass of *Cynodon dactylon, Paspalum paspalodes* and *Sporobolus africanus* for the environmental variables measured

Environmental variables	Grass Species		
	Cynodon dactylon (n=14)	*Paspalum paspalodes* (n=10)	*Sporobolus* (n=8)
Extrac. Phosphate (P)	0.03	0.23	0.27
Extrac Potassium (K)	0.47	0.56* [1]	-0.08
Extrac Calcium (Ca)	-0.49*	-0.52	-0.40
Extrac Magnesium (Mg)	0.78****	0.50	-0.37
Extractable acidity	0.14	-0.42	0.06
pH	0.36	0.29	0.25
Extrac Zinc (Zn)	-0.39	0.22	-0.27
Extrac Manganese (Mn)	0.64***	0.59*	0.17
Organic carbon %	-0.28	-0.54	-0.17
Clay %	0.29	-0.24	-0.04
Moisture	0.12	0.56*	-0.07
Stone content	-0.004	-0.07	0.094
Conductivity	0.15	0.05	-0.07
Methane	-0.50*	-0.16	0.64*
Carbon dioxide	-0.52*	-0.40	0.66*
Oxygen	0.52*	0.21	-0.65*
Temperature	-0.44	-0.58	-0.11
Total biomass of quadrat [2]	0.93****	0.99****	-0.057

[1] Significance level * p<0.1; **p<0.05; ***p<0.02; ****p<0.01

[2] Correlation between the individual species biomass and biomass of all the grasses in the quadrat.

The biomass of *Cynodon dactylon* had a significant positive correlation with the concentrations of Mg and Mn in the soil, p<0.01, p<0.02 respectively (Table 2.5). There were no other significant correlation using the probability level of p<0.05. However, the analysis of the results using the p<0.1 revealed a number of less obvious trends in the data.

Although the level of significance was lower than the commonly accepted limit ($p<0.05$) it provided insight into the possible relationships between the variables measured. Higher concentrations of Ca were found to be negatively correlated ($p<0.1$) with the biomass of *Cynodon dactylon* as found with the pooled species results. The biomass of *Cynodon dactylon* had a significant ($p<0.1$) negative correlation with concentrations of carbon dioxide and methane, however, the biomass increased with increasing concentrations of oxygen ($p<0.1$). The results suggested that *Cynodon dactylon* was growing better in areas with lower carbon dioxide and methane concentrations but higher oxygen concentrations. (Table 2.5). However, the fact that the level of significance ($p<0.1$) for the correlation between biomass of *Cynodon dactylon* and Ca, carbon dioxide, methane, oxygen was very low, limits the interpretation of the correlations to only suggestions rather than reliable conclusions.

Paspalum paspalodes was the only other species to have any significant correlation between biomass and nutrient concentrations in the soil. Concentrations of Mn and K were positively correlated ($p<0.1$) to the biomass of *Paspalum paspalodes* ($p<0.1$). This suggested that variations in the soil nutrients maybe a factor limiting the success of some grass species. The biomass of *Paspalum paspalodes* was also found to significantly increase ($p<0.1$) with the moisture content of the soil, suggesting a possible affinity for moist areas. The level of significance ($p<0.1$) again was very low for the correlation, thus, suggestions rather than conclusions could be made.

Sporobolus africanus had a positive correlation ($p<0.1$) between biomass and carbon dioxide and methane, but a negative correlation ($p<0.1$) with oxygen. This suggested that *Sporobolus africanus* was mainly growing well in areas that had high concentration of

methane and carbon dioxide and low oxygen. This does not necessarily lead to the conclusion that *Sporobolus africamus* preferred these conditions. The reduced competition caused by reduced *Cynodon dactylon* biomass possibly provided an area for establishment.

The correlation between individual species biomass and total biomass gives an indication of the degree to which the species dominates the growth or possibly the amount of competition which the individual species was being exposed to (Table 2.5). *Cynodon dactylon* and *Papsalum paspalodes* have a highly significantly positive correlation ($p<0.01$) with the total biomass, indicating that they are the dominant species in the quadrat. *Sporobolus africamus*, has no significant correlation with total biomass indicating that this species growth is independent of how well other species grow and does not become the dominant species itself (Table 2.5). This lead to the conclusion that *Sporobolus africamus* was perhaps less competitive than the other species but possibly less susceptible to high carbon dioxide and methane, thus allowing the species to grow in areas of higher carbon dioxide and methane.

2.3.2 Bioassay

Any significant difference in growth of the plants between the different soil samples in the bioassay could be attributed largely to soil chemical differences. This would indicate that the cause of the patchy grass growth on the landfill was connected to the chemical factors in the soil and not entirely caused by soil physical structure or landfill gas.

A significant ($p<0.01$) 50% increase in the overall average plant mass was evident for those plants grown in the bioassay for one month. This showed that sufficient growth had

occurred for any acute soil effects on grass growth in the bioassay to be detected. There were no plant mortalities indicating that none of the soil samples taken from the landfill had sufficiently severe toxicity or deficiency of trace elements to result in grass death in 1 month. The root and shoot mass of *Cynodon dactylon* grown in the soil samples from the quadrats in the different areas of each patch (the area without grass; the border grass area; and the surrounding established stand of grass) were compared using an analysis of variance. No significant differences ($p>0.05$) were found in root or shoot mass for any of the soil samples from the four patches investigated. When the data from the four patches were pooled together there was still no significant difference between the different areas from which soil samples were taken (Table 2.6).

Table 2.6: The mean root and shoot weight increase (± standard error) in a 4 week growth period, for *Cynodon dactylon* grown in soil samples from different areas of the four patches on the landfill. (Relative growth was expressed as the weight after 4 weeks minus the original mean weight calculated before bioassay).

Plant material	No grass	Border grass	Established grass	Control (potting soil)
Root mass	0.086±0.021	0.091±0.033 [a]	0.046±0.009	0.131±0.031
Shoot mass	0.200±0.033	0.200±0.034 [a]	0.180±0.037	0.257±0.055

[a] the means in the rows are not significantly different ($p>0.05$) with a Sheffe multiple range test.

These results suggest that soil chemical composition, especially nutrient availability or chemical toxicity by trace elements, was not responsible for the lack of grass growth observed in the four different patches investigated on the Bisasar road landfill. Therefore, the cause for the bare patches on the landfill may be due to one of the variables 'removed' when the soil samples were taken from the landfill, air dried and sieved. These would include changes in the soil atmosphere and in particular carbon dioxide, methane and

oxygen concentrations as well as changes in soil moisture, soil temperature and stone content.

Considering that stone content was not found to vary significantly on the site, nor was it significantly correlated with the differences in grass biomass sampled, it is unlikely to be the cause. The difference in soil moisture was attributed to evaporation due to the lack of grass cover (i.e. a symptom and not a cause of low biomass). Therefore, the bioassay highlighted the importance of the correlation analysis results that suggested that soil gas composition was influencing grass biomass and species distribution and suggested that soil nutrient composition was a less important determinant.

2.4 DISCUSSION

It is important to note that significant differences in the environmental variables measured between the no grass, border grass and established stands of grass do not necessarily identify the reason for the lack of grass growth in any particular patch. Any measured differences maybe due to substrate variation, or maybe as a result of the vegetation growing in the soil thus changing the soil characteristics. However, the comparison of the levels of the variables measured with normal soil conditions, as well as with the result of the bioassay, would help confirm the role these environmental variables had in affecting grass establishment and growth. The results provide an indication of which environmental variables do vary on the landfill and their possible relationship with grass distribution.

Twelve different species of grass were identified on the Bisasar Road Landfill site. This is similar to the grass species diversity found on the Gin Drinkers' Bay Landfill, Hong Kong on which 10 different grass species were recorded (Wong & Yu, 1989). A wide range of

cover materials used on landfills will influence the cover and number of species, and may result in high plant species richness (Ettala *et al* 1988). The types of waste underlying the cover material, which produce different amounts of landfill gas and leachate, may also cause variation in the soil, which also influences species colonisation and distribution.

Out of the twelve species, *Cynodon dactylon, Paspalum paspalodes* and *Sporobolus africanus*, in terms of relative abundance, were the most successful colonisers of this area of the landfill. The micro-distribution of the species around bare patches provided insight into the performance of the species in relation to possible spatially variable soil conditions. *Cynodon dactylon* was the predominant and most competitive species in the established stands, forming an almost complete monoculture. However, it appeared to be sensitive to the environmental variables causing the bare patches and was relatively less abundant in the border areas of the bare patches. The opposite was apparent for *Paspalum paspalodes* and *Sporobolus africanus*, although, not as widely distributed as *Cynodon dactylon*, these species were predominantly found in the border areas of the bare patches.

In the established grass stands the environmental conditions were sufficient to support a large standing biomass of *Cynodon dactylon* and competition between species was probably a major factor determining the distribution of other species. The reduced biomass in the border areas of the bare patches resulted in lower levels of competition and an opportunity for other species to colonise, thus resulting in a higher species richness. However, the species that colonised the border areas would have to be less sensitive to the environmental conditions causing the bare patches than *Cynodon dactylon*. Therefore, *Paspalum paspalodes* and *Sporobolus africanus*, which were the most successful colonises of the

border areas, probably had the greatest relative tolerance to the environmental conditions causing the bare patches.

Lower levels of soil K, Mg and moisture were associated with the bare patches in which no grass would grow. However it was difficult to determine if these were causal factors. Deficiencies of K and Mg in the soil can limit vegetation growth (Munshower, 1994). However, the Mg levels in the bare areas and the established stands were within the normal ammonium acetate extractable range for soils, of 40 – 500 mg Kg^{-1} (Grimshaw *et al* 1989). Magnesium although a macronutrient is also only needed in relatively small quantities by plants and, therefore, it is not usually in short supply (Bradshaw & Chadwick, 1980). The lack of any significant differences in plant biomass in the bioassay was also a clear indication that the variability in K and Mg within the soil was unlikely to be the primary cause of the bare patches. In terms of soil moisture, it is difficult to determine if the low soil moisture in the bare areas was a cause or an effect of no grass cover. There was no apparent physical difference in the soil structure, as shown by the stone and clay contents. Therefore, the lower moisture levels in the bare areas were most likely due to increased evaporation from the soil caused by the lack of protection from a vegetation canopy and the higher surface temperature. Thus a lack of soil moisture in areas of the landfill was unlikely to be the cause of patchy grass growth.

High levels of soil Ca and Zn were associated with the bare areas on the landfill, however, again these results do not necessarily show a causal relationship. There is little concern with regards to soil calcium deficiency or excess unless soil pH extremes are apparent (Munshower, 1994). In this investigation the soil pH was not extreme and ranged from, 7.4 - 8.1, therefore, it was unlikely that the Ca levels were directly responsible for the lack of

grass growth. However, elevated Ca levels can influence the availability of essential trace metals, especially Mn and Fe, thus possibly resulting in plant deficiencies (Grimshaw *et al*, 1989). High levels of soil Ca are also generally associated with leachate contamination and are one of the pollutant ions mainly responsible for increased soil salinity (Hernandez *et al* 1999). This may explain the relatively high soil conductivity values recorded throughout the study area.

High soil conductivity as a result of leachate contamination has been shown as the cause of poor vegetation growth on some landfills (Hernandez *et al* 1999; Lan & Wong, 1994; Wong *et al* 1992). However, the contamination of soil with leachate can also be beneficial for plant growth as it can provide much needed moisture and nutrients (Cureton *et al* 1991; Gordon *et al* 1989). Although the mean soil conductivity in this investigation was 5.5 mS cm^{-1} which is above the recommended level, of 2 mS cm^{-1}, for non-tolerant vegetation growth (Bradshaw & Chadwick, 1980; Gilman *et al* 1985; Moffat & Bending 1992), there was no apparent relationship between soil conductivity and the lack of grass growth. The dominant species on the site, *Cynodon dactylon*, has been reported to be leachate tolerant and is commonly used for the reclamation of landfills (Menser *et al* 1979, 1983) and Tong & Wong 1984, showed that *Cynodon dactylon* seed germination was improved by low concentrations of leachate irrigation. Therefore, the natural colonisers of the site appear to be tolerant of leachate contaminated soils and leachate was unlikely to be the cause of the bare areas. Again, if high levels of soil Ca or conductivity were responsible for the bare patches a significant difference in plant biomass in the bioassay would have been expected.

In terms of soil Zn, toxicity is only usually found in soils with a pH below 5.5 (Pais & Jones, 1997). The lowest soil pH measured in this experiment was 7.4.

Diethylenetriaminepentaacetic acid (DTPA) extracted soil zinc reveals a phytotoxic response between 50 and 125 mg kg^{1} (Munshower, 1994). Although, a different extracting solution was used in this investigation, the highest zinc level measured was 27mg kg^{-1} which was considerably below the levels reported to be phytotoxic. Therefore the relationship seen between no grass growth and soil Zn levels was also unlikely to be the key reason for the bare patches.

The data showed an unclear relationship between grass growth and the soil Mn and P levels. However, considering soil P toxicity in the natural environment is unknown and low levels of P are usually the limiting factor (Munshower, 1994), it is unlikely that the high levels of P in the bare areas can be responsible for the lack of grass growth. Leachate contamination of the soil can result in increased Mn concentrations (Lan & Wong, 1994; Winant *et al* 1981), and is often associated with poor vegetation growth (Lan & Wong, 1994; Winant *et al*, 1981, Wong & Yu, 1989). However, Wong and Yu, (1989) found Mn concentration to have a significant negative correlation with forb growth but not grasses, suggesting a possible greater tolerance of grasses. The normal soil range for (ammonium acetate) extractable Mn concentrations is 5 - 500 mg kg^{-1} (Grimshaw *et al* 1989). The established stands of grass in patches 1 and 2 (Table 2.3) had significantly higher Mn concentrations by comparison to the bare areas, however, the concentrations were within the normal soil range (Grimshaw *et al* 1989). Manganese is usually only toxic when the soil pH is low (<5.5) or under strong reducing conditions such as that found in anaerobic soils (Munshower, 1994; Pais & Jones, 1997; Winant *et al* 1981). In this investigation the lowest pH recorded was 7.4 and lowest oxygen level was 8.5%. Therefore, these soils did not have a low pH and were not anaerobic, thus, Mn was unlikely to be toxic. Wong & Yu (1989) found significantly higher extractable Mn concentrations on the Gin Drinker's Bay Landfill,

Hong Kong, by comparison to an off-site control area which had similar soil total Mn levels. The results found by Wong & Yu (1989) and the variation in Mn in relation to the bare areas on the landfill suggest the need for further investigation into this aspect of landfill soil chemistry. It is difficult to get an accurate extractable Mn concentration as the *in situ* redox potential of the soil is difficult to maintain once soils are sampled, thus influencing the availability of Mn.

There was evidence in the correlation analysis to suggest that soil temperature, pH and elevated CO_2 may be responsible for the lack of grass growth in the bare patches. However, the higher soil temperature associated with the bare patches is more likely to be a result of the lack of vegetation, as with moisture, than a cause. Especially considering that the soil structure did not appear to vary significantly within the study site. The pH range found in this study was within the normal range of 4.5 - 8 recommended by McKendry, (1996) for soils used in landfill restoration. Therefore, pH was also unlikely to result in the total lack of grass growth in certain areas and a significant effect on plant growth would have been apparent in the bioassay. The remaining variable that had an apparent association with the bare areas in the study area was elevated soil CO_2. Although the soil gases in the no grass areas and the established stands were both in excess of what would be expected for healthy soils, the correlation analysis suggested a possible relationship between higher soil CO_2 and the lack of grass growth.

From the results of the bioassay and the discussion of the soil chemical data above, landfill gas infiltration into the soil appeared to be the most likely variable responsible for poor grass growth. The concentrations of carbon dioxide ranged from 0 - 39 %, with the lower figures being associated with higher grass biomass (Figure 2.2). The normal range of carbon

dioxide concentrations in the soil atmosphere is 0.1% - 5% (Geisler, 1963; Gendebien *et al* 1992), therefore, the areas on the landfill with carbon dioxide concentrations in the upper part of the range measured were probably exposed to considerable landfill gas infiltration.

Similar research on the bare patches found on landfills has been conducted by a number of other workers (Chan *et al* 1991; Lan & Wong 1994; Wong & Yu, 1989; Wong *et al* 1992; Wong, 1988). The concentrations of all the three gases (methane, carbon dioxide and oxygen) were not always measured, therefore, it is difficult to conclude which gas influenced vegetation the most. However, generally the lower the vegetation cover and plant survival the greater the reported methane and carbon dioxide levels and lower the oxygen levels. As in this investigation, Wong & Yu, (1989) found no significant correlation between the vegetation performance and methane concentrations. Methane does not appear to exert any direct effect upon vegetation, but does reduce the amount of oxygen in the soil by displacement (Chan *et al* 1991, Ettala *et al* 1988, Flower *et al* 1981). However, in this investigation the oxygen levels ranged between 9% - 18% and only when oxygen levels are below 10% are plants usually affected (Flower *et al* 1981). Therefore, it was unlikely that the oxygen levels in the bare areas were responsible for the lack of grass growth. Unlike, methane and oxygen, the relationship between poor grass growth and carbon dioxide was more likely. A significant negative correlation between carbon dioxide and vegetation cover was also found by Chan *et al* (1991) and Wong & Yu, (1989) strengthening the conclusion that the levels of carbon dioxide in the bare areas on the Bisasar road landfill was probably a key variable limiting grass growth.

It is interesting to note that Chan *et al* (1991) measured 82% vegetation cover in an area with a mean carbon dioxide concentration of 17.6 % and mean oxygen concentration of

9.7%. However, on the Bisasar Road landfill, the totally bare areas of the site had lower soil carbon dioxide concentrations and higher soil oxygen conditions. Wong *et al* (1992) measured very similar carbon dioxide (15.1%) and oxygen (12.7%) concentrations, as found on the bare areas in this investigation, in an area of the Gin Drinkers' Bay Landfill with a 33% vegetation cover. A possible explanation for the presence of vegetation in these high gas areas may be attributed to species tolerance. Although, not discussed by Chan *et al* (1991) or Wong *et al* (1992), their results showed the grass *Panicum repens* as the most predominant species on their site, accounting for the majority of the cover measured in the high gas areas. *Panicum repens*, appeared to be a relatively more tolerant species to landfill gas than other species on the Gin Drinkers' Bay Landfill and possibly more tolerant than the species found on the Bisasar Road landfill. It must also be pointed out that the gas measurements made on the Bisasar Road landfill did not account for any temporal variation in gas concentrations that may occur. Therefore higher peak levels of soil carbon dioxide and methane and lower oxygen levels than that measured here, could possibly occur in the bare patches.

Cynodon dactylon, which was the predominant species on the Bisasar Road Landfill, was one of the relatively less common species found by Chan *et al* (1991) and Wong *et al* (1992) and it had a very low cover in the high gas areas. This corresponded with the correlation analysis for the individual species biomass in this investigation (Table 2.5) which showed, although at a level of significance $p<0.1$, a negative correlation between *Cynodon dactylon* biomass and methane and carbon dioxide levels. This suggested that although, *Cynodon dactylon* is tolerant to leachate contaminated soils (Bradshaw & Chadwick, 1980; Menser *et al* 1979, 1983; Tong & Wong 1984), it was sensitive to carbon dioxide and possibly methane levels in the soil.

Although, not discussed by Lan & Wong, (1994) and Wong *et al* (1992), their results showed that *Paspalum sp.* was found mainly in the high gas areas in comparison to lower gas areas. On the Bisasar Road Landfill, *Paspalum paspalodes* was predominately in the border areas of the bare patches, also showing a possible tolerance of the species to landfill gas. However, *Paspalum paspalodes* usually colonises moist areas (Gibbs Russell *et al* 1990) and will probably perform best when conditions are moist, as indicated by the positive correlation between moisture and biomass of this species (Table 2.5). *Sporobolus africanus* has not been found in any other investigations on landfills but the colonisation of the border of the bare areas would suggest relatively higher tolerance of this species to high carbon dioxide concentrations.

Elevated carbon dioxide levels in the soil probably presents the greatest factor limiting grass growth on the landfill. Gas extraction is an expensive and not always successful solution, therefore, the selection of species more tolerant to the conditions is probably a worthwhile solution (Flower *et al* 1981). *Cynodon dactylon* is a good species for revegetation of landfills (Menser *et al* 1983; 1979), however, the possible greater sensitivity to elevated soil carbon dioxide and methane and reduced oxygen levels by comparison to other grass species suggests that other more suitable species may be available.

The use of the *Panicum repens* which appears to colonise areas of similar and higher soil atmosphere gas concentrations, on other landfills, may be a potential solution. *Panicum repens* has a broad distribution in southern Africa and is often found in wet sandy soils, sometimes adjacent to either a fresh or brackish water sources. The species is good for erosion control and is often planted around dams in Zimbabwe (Gibbs Russell *et al* 1990). The results of this investigation indicate that *Sporobolus africanus* and *Paspalum*

paspalodes are also promising species. However, a better understanding of the mechanisms by which landfill gas infiltration limits grass colonisation and growth needs to be attained, thus, facilitating the screening of grass species and possible treatment of the site to improve the success of landfill revegetation.

CHAPTER 3: TREE GROWTH AND SURVIVAL: A PRELIMINARY FIELD INVESTIGATION

3.1 INTRODUCTION

In October 1995 Durban Solid Waste decided to plant trees on the main stability berm of the Bisasar Road landfill, in order to create a rising "green wall" as the landfill site developed. The stability berm already had a grass layer but had extensive erosion. Trees were introduced so as to provide more stability and improve the aesthetics of the site. It was noted by Durban Solid Waste that the trees planted were growing very slowly and a large number had died. This is a commonly found problem with landfill revegetation, especially when trees are used (Chan *et al* 1991; Lan & Wong, 1994; Dobson & Moffat, 1994). However, the use of species that are tolerant to the conditions on landfills can improve the success of revegetation (Flower *et al* 1981; Robinson *et al* 1992).

Although the trees on the stability berm of the Bisasar Road landfill were not planted for research purposes they held the only available information, to our knowledge, regarding South African indigenous tree growth and survival in a landfill environment. The investigation into the health of the different trees species planted and the environmental conditions on the stability berm of the landfill site would provide important information regarding the types and extent of the challenges presented to trees and how they respond.

The results of this preliminary investigation would then allow for further investigations to be more focused on the environmental variables which present the greatest problem and the development of an experimental screening procedure for tree species selection with regard to possible greater tolerance to these conditions.

3.2 MATERIALS AND METHODS

3.2.1 Site description

The main stability berm is situated on the northern side of the landfill site at the bottom of the valley (Figure 1.2). It stretches across the base of the valley and rises to a height of 24 m with a gradient of 1 : 2 and is approximately 230 m wide (Figure 1.2). This stability berm forms the first terrace consisting of the wastes that were first deposited when the site opened in 1980. The front face of the berm is made up of building rubble, rocks and carbonaceous shale covered with a thin layer of various soils. The front face of the berm had been planted with *Cynodon dactylon* and several bands of *Vetiveria zizanoides* (Vetiver grass) to stabilise the slope.

3.2.2 Investigation of the trees planted on the stability berm

In October 1995, an unequal number of twenty different indigenous tree species, approximately 1.5m in height, were planted on the slope of the berm, totalling 210 trees. There was no pre-treatment of the soil on the berm and the trees were planted, at an even distribution across the berm, with only the soil from their potting bags surrounding their roots. The trees received no aftercare, such as watering or weeding.

Surface run-off of rain from the completed section of the landfill above the berm, during the period between October 1995 and February 1996, resulted in extensive erosion of the central section of the stability berm. An unknown number of trees were washed away and destroyed by earth moving machinery used to repair the erosion damage.

A survey of the trees on the berm was conducted in May 1996 so as to determine the actual

numbers of each of the species remaining. The different tree species were identified and the relative position of each of the trees was recorded on an aerial photograph of the stability berm with the aid of an overlaid grid system. Stem diameters were measured 5cm from the ground with digital caliper and the tree height was measured with a steel tape from the ground to the highest shoot. The stem diameter and tree heights were measured for comparison to further measurements to be taken later in the year. All the trees were tagged and their condition was recorded on the 6 May and the 1 August 1996 according to a set of health categories shown in Table 3.1. Although these categories (Table 3.1) were subjective, the classification of the health of the trees on each occasion were completed by the author during a single day so as to reduce possible bias in the results

Table 3.1: Tree health categories based on the general appearance of the trees

Category	Description
1	Very healthy: Full set of leaves with the majority of the leaves not showing any discoloration or chlorosis. Overall good condition with signs of new growth.
2	Healthy: Full set of leaves, however majority showed some signs of discoloration and / or chlorosis. New shoots were present.
3	Poor health: Less than 30% loss of leaves. Leaves remaining maybe discoloured but with majority of leaf area still green. The stems and branches were still flexible and not showing signs of drying out. New shoots were present.
4	Unhealthy: Greater than 40% loss of leaves. Leaves were brown or browning with very little green remaining. Sections of the tree were dead (as described in category 5). No new shoots present.
5	Dead: No leaves, the remaining stem and branches were dry and brittle, no moisture in any of the plant material remaining.

3.2.3 Environmental variables measured on the stability berm

Measurements of the environmental variables were made so as to characterise the general conditions to which the trees were exposed to on the main stability berm. Environmental conditions on landfill sites can have large spatial variation, especially landfill gas (Dobson & Moffat, 1994; Wong *et* al, 1992), therefore, the environmental conditions surrounding each individual tree were measured. The following environmental variables were measured in the soil surrounding the trees: methane and carbon dioxide concentrations in the root zone; soil pH; soil stone content; and % soil moisture.

Landfill gas in the root zone of the trees was of the greatest interest. Therefore, a probe for sampling landfill gas in the root zone needed to be developed. The length of the probe was determined by the depth of the root zone. Considering that adult trees seldom have roots deeper than 1.5m (Dobson & Moffat, 1994), and the trees in question were not adult but only 1.5m in height (whips), the root zone was assumed to be less than 1m below the soil surface. This was confirmed by the excavation of several trees of different species on the stability berm, which were found to have their main root mass not much deeper than 0.45m. The design of a probe was based on that outlined by others (Barry, 1987; Chan *et al*, 1991; Lan & Wong, 1994; Lombard and Associates, 1994; Wong *et al*, 1992). The following design for the probe was utilised: 1m lengths of 24mm outer, 16mm inner diameter plastic water pipe was used. The bottom 35cm was drilled with 5mm holes, 5cm apart to allow for the gas in the root zone to migrate into the probe (Figure 3.1). The top of the pipe was capped with a plastic airtight stopper to prevent dilution of the landfill gas by direct atmospheric exposure. The probes were positioned randomly within a 0.5m radius of the stem of each tree, but in such a way as not to damage the tree, and were inserted to a depth of 50cm below the soil surface. The probes were all inserted to the same depth as gas

concentrations are also found to vary with soil depth (Dobson & Moffat, 1994).

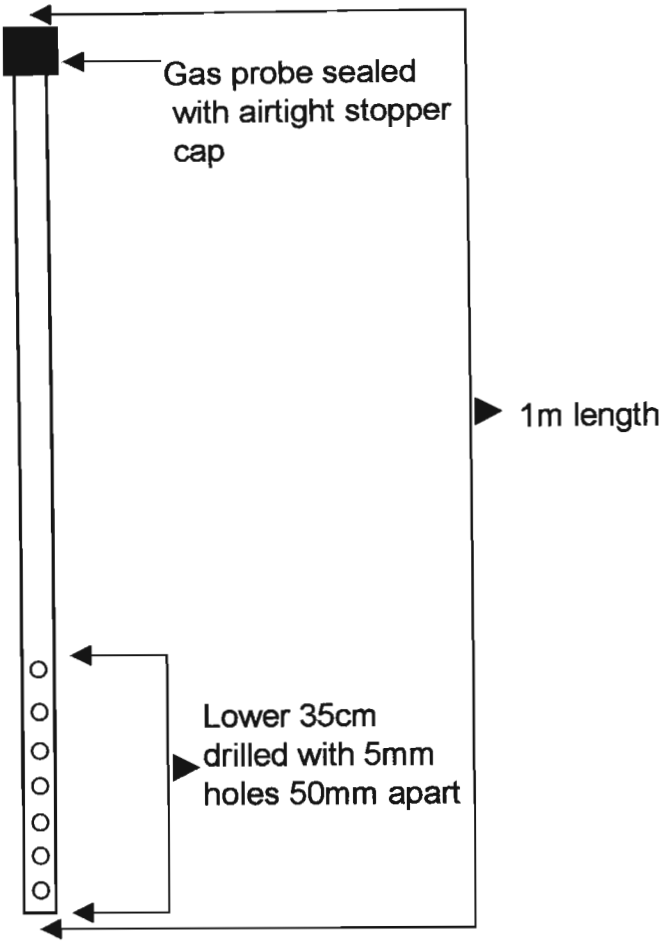


Figure 3.1: Gas probe made of 24mm plastic tube, used for sampling landfill gas within the root zone

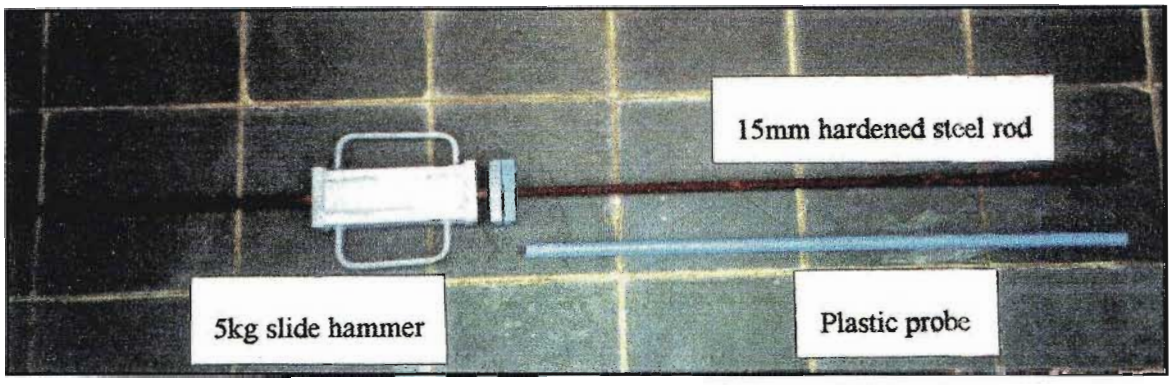


Figure 3.2: A sharpened 15mm diameter hardened steel rod onto which the probe could slide was fitted with a 5kg slide hammer, this was used to insert the probes into the hole created by the dynamic cone penetrometer (D.C.P).

A device for inserting the probe into the ground needed to be constructed. As suggested by Barry (1987), a steel rod was driven into the ground using a slide hammer to make the hole for the probe to be inserted. However, upon removal of the steel rod the hole became clogged with stones and loose soil making it impossible to insert the probes. So as to overcome this problem a sharpened 15mm diameter hardened steel rod, fitted with a 5kg slide hammer, onto which the plastic probe could slide was constructed (Figure 3.2). Using a dynamic cone penetrometer (D.C.P.) with a 50mm diameter head, a hole was driven into the ground. The D.C.P. was then removed and with the support of the hardened steel rod the plastic probe was driven into the hole. Once the steel rod was removed the probe was sealed with a plastic cap and the surrounding loose soil was packed tightly against the sides of the probe to prevent gas escaping. Gas samples were taken from the probes (which remained in the ground) at intervals for the duration of the investigation.

Although methane is less dense than air, when it is mixed with other landfill gases and diluted it may not be very buoyant unless there is a considerable temperature or pressure difference (Barry, 1987). It is for this reason that an aspirator must be used for the removal of a sample of gas from the probes. Gas samples taken from the probes can be analysed using a gas chromatograph but this is an expensive procedure, especially when considering the large number of probes and replicated measurements required for this investigation. The availability and expense of a portable carbon dioxide and oxygen meter also presented a problem. However, for this investigation a portable methane meter (G624p Exotector) with a built in aspirator was available. The methane meter determined the percentage methane by the thermal conductance characteristics of the gas sample taken. Methane is not directly toxic to plants, but methane concentrations can be regarded as an indirect indicator of concentrations of more important gases i.e. high carbon dioxide and low

oxygen (Chan *et al*, 1991). For comparison with the methane concentrations measured in the root zone, a portable carbon dioxide meter (Draeger Multiwarn, Infrared CO_2 0-100%, No. 6807940) was used for CO_2 measurements, however, it was only available for a limited period.

Methane was measured either in the early mornings, at midday or in the late afternoon on five separate days. Gas measurements were taken once a day so as to ensure that gas concentrations in the probe had time to equilibrate with the root zone soil atmosphere. Variations in temperature, rainfall and barometric pressure tend to influence landfill gas emissions (Chan *et al*, 1991; Dobson & Moffat, 1994; Lombard & Associates, 1994). Therefore, temperature and barometric pressure were recorded with each set of gas measurements made. Carbon dioxide was measured on only two separate occasions due to the availability of equipment. The mean value for the gas concentration from each probe was compared with data from all the probes in order to locate areas with high landfill gas on the stability berm. The gas concentration measurements were also compared to the relative health of the trees.

Soil samples were collected using a soil auger from the top 15cm to 20cm of soil. Two auger samples were taken randomly within a 0.5m radius of each tree. The two soil samples were immediately sealed into a polythene bag and mixed. The samples were transported back to the laboratory where pH, stone content, and moisture content were determined.

The pH of the fresh soil samples was measured using a pH electrode (Hach Model 43800) with a 1:1 ratio of soil to distilled water (Grimshaw, 1989). Any large stones were removed

from the samples for pH analysis so as to prevent damage to the electrode. pH measurements were replicated three times for each soil sample. For the analysis of stone content the soil samples were air dried at room temperature (Grimshaw, 1989) and the soil aggregates were gently broken up with a mortar and pestle. The sample was separated with a 2mm sieve dividing the soil from the 'stone' (>2mm fraction). The weight of stones was then expressed as a percentage of the total weight of the soil sample that was sieved. The moisture content of the fresh soil samples were measured as described by Grimshaw (1989). 10-20 g fresh soil samples, with large stones and roots removed, were weighed in dry evaporation basins. These samples were placed in an air circulation oven at 105°C until they reached a constant weight. They were then cooled in a desiccator and the percentage fresh moisture was calculated from the loss in weight. Each sample was replicated three times.

Statistical analysis of the data collected was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data were analysed using an analysis of variance. If there was a significant difference ($p<0.05$) in data with more than two sample variables then Scheffe multiple range test was performed by constructing intervals for pair-wise differences of means to determine which differences were significant ($p<0.05$).

3.3 RESULTS

3.3.1 The trees on the stability berm

A total of 210 trees comprising of twenty different species were listed to have been planted on the main stability berm (Table 3.2). All of the trees that were planted were staked into the ground (D. Dorkin, 1996 *pers comm*), therefore, although the trees may have died the

stakes would still remain indicating the position of the tree. However, the survey completed in May 1996 revealed that only 110 trees alive or dead were present (Table 3.2). An average of only 47 % of the total individuals of each species initially planted was actually found with some species not being found at all.

Table 3.2: A list of the tree species planted in October 1995 on the main stability berm and the numbers of these trees found in the survey carried out in May 1996.

Species	No. supplied	No. recorded in survey
Acacia sieberiana	12	7
Acacia xanthophloea	4	4
Celtis africana	13	6
Combretum erythrophyllum	20	10
Cussonia spicata	8	0
Dais cotinifolia	10	0
Dombeya rotundifolia	8	3
Erythrina lysistemon	11	8
Harpephyllum caffrum	12	3
Heteropyxis natalensis	6	4
Hibiscus tiliaceus	4	3
Peltophorum africanum	4	2
Rhus lancea	17	14
Schotia latifolia	8	5
Schefflera umbellifera	2	0
Strelitzia nicolai	20	2
Syzygium cordatum	30	28
Tabernaemontana ventricosa	11	0
Trema orientalis	6	4
Ziziphus mucronata	4	0
Support stakes without trees [1]	--	7
TOTAL	210	110

[1] All the trees planted were staked into the ground, therefore, although trees may have died the stakes could remain, indicating the position of the tree.

Since October 1995 considerable erosion of the central portion of the stability berm had taken place and earth works were completed so as to repair this damage. This operation

and the erosion probably accounted for a large number of trees being destroyed.

Due to the low numbers of each tree species the health category system (Table 3.1) was simplified, as explained in Table 3.3. The condition of each species was expressed as a proportion of healthy trees of that species found on the berm (Table 3.3).

Table 3.3: Proportion of the trees of each species found on the stability berm which were healthy in May 1996[1]

Species	Proportion healthy [1]	No. of trees of each species
Strelitzia nicolai	1	2
Harpephyllum caffrum	1	3
Acacia xanthophloea	1	4
Rhus lancea	0.68	14
Hibiscus tiliaceus	0.67	3
Combretum erythrophyllum	0.55	10
Acacia sieberiana	0.5	7
Peltophorum africanum	0.5	2
Schotia latifolia	0.5	5
Celtis africana	0.42	6
Heteropyxis natalensis	0.38	4
Dombeya rotundifolia	0.33	3
Syzygium cordatum	0.29	28
Trema orientalis	0.25	4
Erythrina lysistemon	0	8

[1]Proportion of healthy trees calculated using a simplified version of the health ranking system (Table 3.1). Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy. Trees that were ranked as 3 were divided and 0.5 was added to the healthy and unhealthy groups. Thus, proportion healthy = [(No. of trees ranked 1 & 2)+(No. of trees ranked 3 x 0.5)] ÷ (Total number of trees of the species)

All the *Strelitzia nicolai, Harpephyllum caffrum* and *Acacia xanthophloea* trees on the stability berm were 'healthy'. *Rhus lancea, Hibiscus tiliaceus* and *Combretum erythrophyllum* had predominantly 'healthy' trees growing on the berm. *Peltophorum*

africanum, *Schotia latifolia*, and *Acacia sieberiana* had the same proportion of 'healthy' and 'unhealthy' trees growing on the berm. However, *Trema orientalis*, *Syzygium cordatum*, *Dombeya rotundifolia*, *Heteropyxis natalensis* and *Celtis africana* had low proportions of healthy trees. No 'healthy' trees of *Erythrina lysistemon* were found on the stability berm in May of 1996. It must be noted that for some species with low numbers of individuals, the proportion of 'healthy' individuals may not accurately represent the species performance under landfill conditions. There is often large spatial variation in the environmental conditions on a landfill, therefore, the smaller the number of trees, the greater the chance that all the trees of one species trees may have only been planted in either, an exceptionally harsh or, a favourable area of the berm.

For further analysis of these data, the number of species was reduced to seven species that had greater than 5 individuals, allowing for a more focused investigation of the individual species in relation to the environmental variables measured. The selection of the species was further reduced to five, that is those species which were ranked predominately 'very healthy' (category 1), namely *Rhus lancea*, *Combretum erythrophyllum*, *Acacia sieberiana*, or 'dead' (category 5), namely *Syzygium cordatum* and *Erythrina lysistemon*. This was done as the number of individuals within each health category for *Celtis africana* and *Schotia latifolia* was too low for meaningful results to be obtained.

The health category measurements made on these five tree species in May were repeated in August (Table 3.4). A comparison of the measurements between May and August showed, that unlike the other four species, *Erythrina lysistemon* had a marked increase in the proportion of healthy trees. In May *Erythrina lysistemon* was classified as unhealthy because of its lack of leaves when this was in fact probably a seasonal effect. By August

Erythrina lysistemon was no longer dormant and began to grow, thus, the August health measurements provided a better representation of the condition of the species. For the other two deciduous species, *Acacia sieberiana* and *Combretum erythrophyllum*, there was no improvement in health between May and August, suggesting that the health of these species was accurately observed and the results were not affected by seasonal changes.

For the non-deciduous species, *Syzygium cordatum* and *Rhus lancea,* there was unlikely to be any seasonal influence, therefore, the proportion of healthy trees probably provided a good representation of health condition of the species. The proportion of healthy trees of *Syzygium cordatum* was much lower in August by comparison to April showing a deterioration in tree health (Table 3.4), whereas *Rhus lancea* had very little change in the proportion of healthy trees. It must be noted that further observations of the trees over a longer period of time, preferably more than one season, would have provided a better indication of the performance of the species.

In order to obtain estimates of species growth rates stem diameter and tree height were recorded in May 1996, by comparison with measurements to be made later in the year. However, due to the unforeseen construction of a rainwater drainage pipe down the centre of the stability berm and a gas reclamation pipeline diagonally across the stability berm in October 1996, 30% of the trees measured in May on the stability berm were destroyed. The number of replicates for each tree species became too low for the growth rate results to have any statistical validity and therefore, this study was abandoned.

Table 3.4: The proportion of healthy[1] trees for the five tree species in May and August 1996 and the most likely reason for the change (health effect: change due to deteriorating health of tree; Seasonal effect: change due to tree emerging from winter dormancy).

Species	May	August	Possible reason for change
Deciduous species*			
Acacia sieberiana (n=7)	0.5	0.36	Health effect
Combretum erythrophyllum (n=10)	0.55	0.2	Health effect
Erythrina lysistemon (n=8)	0	0.63	Seasonal effect
Non deciduous*			
Syzygium cordatum (n=28)	0.29	0.09	Health effect
Rhus lancea (n=14)	0.68	0.64	Little change

[1] Proportion of trees healthy calculated using a simplified version of the health ranking system (Table 3.3). Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy. Trees which were ranked as 3 were divided and 0.5 was added to the healthy and unhealthy groups. Thus, proportion healthy = [(No. of trees ranked 1 & 2)+(No. of trees ranked 3 x 0.5)] ÷ (Total number of trees of the species)

* As described by Palgrave, 1984

3.3.2 Environmental variables

The mean percentage methane in air recorded within the root zone on the stability berm was 13.6 (std error 1.2) with a large range between 0 and 60%. The mean percentage carbon dioxide in air within the root zone was 4.2 (Std error 0.5) with a minimum of zero and a maximum of 22%. The mean carbon dioxide and mean methane measured at each probe had a significant ($p<0.05$; $R^2 = 0.63$) linear relationship, with carbon dioxide increasing with methane concentrations. There was no significant variation ($p>0.05$) in methane concentrations measured in the early morning, midday or late afternoon. There was also no significant ($p>0.05$) variation in methane measured at different barometric pressures and temperatures, however, the range of atmospheric temperature (18°C - 29°C) and pressure (1024 mb - 1039 mb) was relatively small.

The health measurements made in August were used for comparison with the environmental variables measured. However, the health category system (Table 3.2) was again narrowed down from five categories into two: healthy; and unhealthy (i.e. Trees ranked 1 and 2 were classified as healthy, those ranked 4 and 5 were classified as unhealthy, as shown in Table 3.3. The individual trees ranked as 3 were not divided and 0.5 added to the healthy and unhealthy categories for the species, as done before. The health classification of individuals, in August, with a health rank of 3, was determined by the change in health of the individual tree between May and August. If the health of the individual tree had deteriorated between May and August it was classified as unhealthy and *visa versa* for those individuals put into the healthy category.

The comparison of the health of the trees (all species combined) on the stability berm with the mean methane concentrations measured in the root zone, showed that the trees classified as unhealthy had significantly ($p<0.05$) higher root zone methane concentrations by comparison to the healthy trees. Similarly, the analysis of the root zone methane concentrations for the individual species gave the same conclusion, with a significantly ($p<0.05$) higher methane concentration in the root zone of the unhealthy trees of each species, except for *Erythrina lysistemon* (Figure 3.3). *Erythrina* had no statistically significant ($p>0.05$) difference in methane concentrations between healthy and unhealthy trees. It is important to note that the numbers of individuals within one of the two health categories was often very low, especially for *Acacia sieberiana* and *Syzygium cordatum*, thus, limiting the interpretation of the results (Figure 3.3). However, the results suggest that concentrations of methane in the root zone were related to the health of the trees. Out of the five species, the health of *Erythrina lysistemon* appeared to be the least affected by the methane in the root zone.

Figure 3.3 shows that not all of the species were exposed to the same average methane concentrations. This can be attributed to the large spatial variation in the landfill gas concentrations on the stability berm. It is important to note that the healthy trees of *Acacia sieberiana*, *Syzygium cordatum* and *Combretum erythrophyllum* were found in areas of very low methane (< 2%) concentration. Whilst, the healthy trees of *Rhus lancea* and *Erythrina lysistemon* were found in areas of considerably higher methane, 9% and 20 % respectively. This suggests that *Erythrina lysistemon* and *Rhus lancea* were less susceptible to higher methane concentrations in the root zone by comparison to the other three species. However, the low numbers of individual trees for each species in most of the two health categories indicate that these conclusions should be treated with caution.

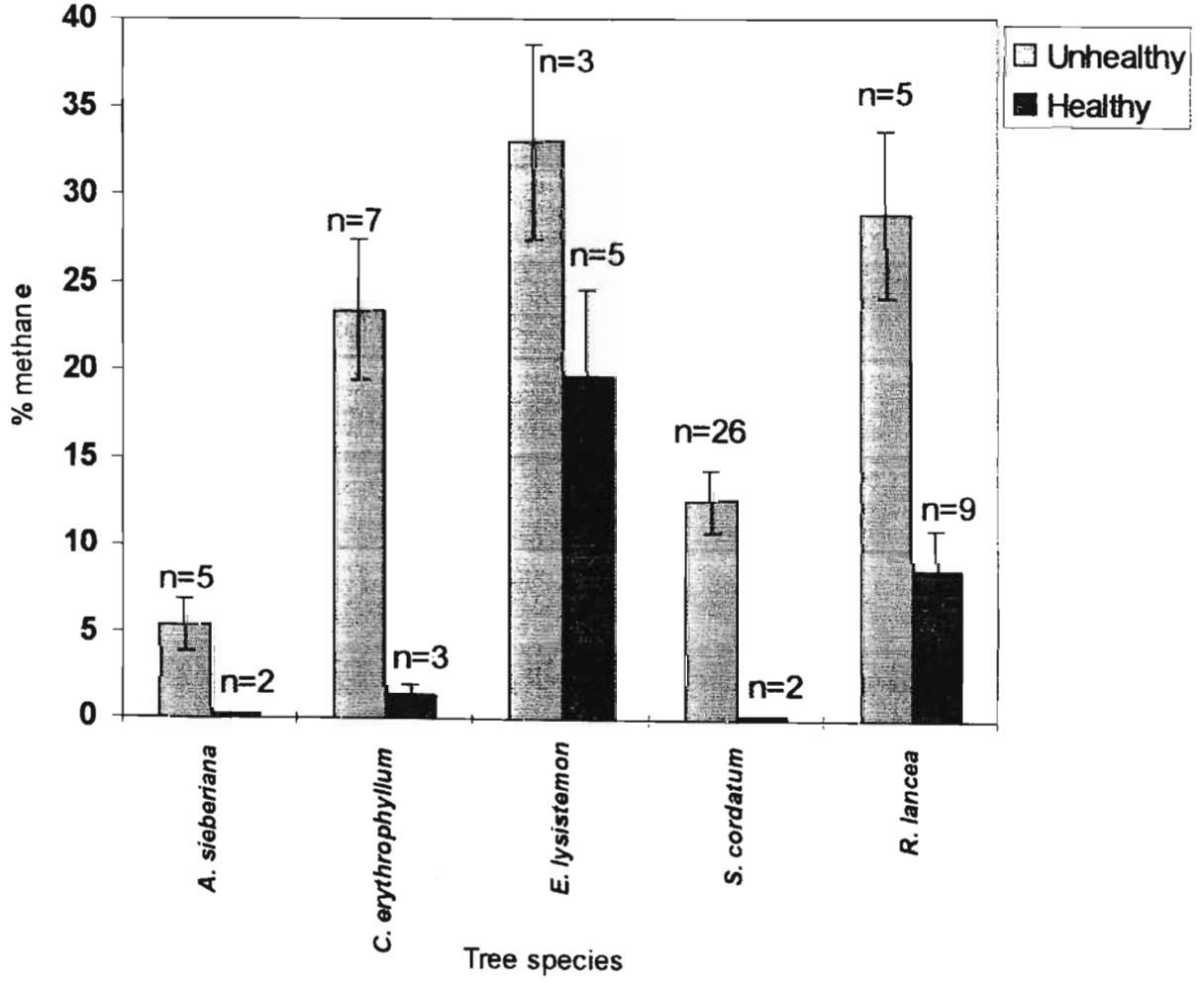


Figure 3.3: The relationship between methane in the root zone and species health in August 1996. Results are mean values with standard errors.

The analysis of the soil on the stability berm showed that the mean pH was 6.42 (Std error 0.05) with a minimum value of 4.8 and a maximum of 8.0. There was no significant ($p>0.05$) difference in soil pH between the healthy and unhealthy trees on the stability berm. The same was the case for the analysis of the soil pH for the individual species, except for *Acacia sieberiana*. The unhealthy trees of *Acacia sieberiana* had a significantly ($p<0.05$) higher soil pH when compared to the healthy trees of the same species (Figure 3.4). However, the pH was not significantly ($p>0.05$) higher than the pH conditions that the other four species were exposed to. The significant difference in *Acacia sieberiana* soil pH may suggest that species preferred lower soil pH. The results generally suggested that soil pH was probably not one of the main variables influencing the health of the trees.

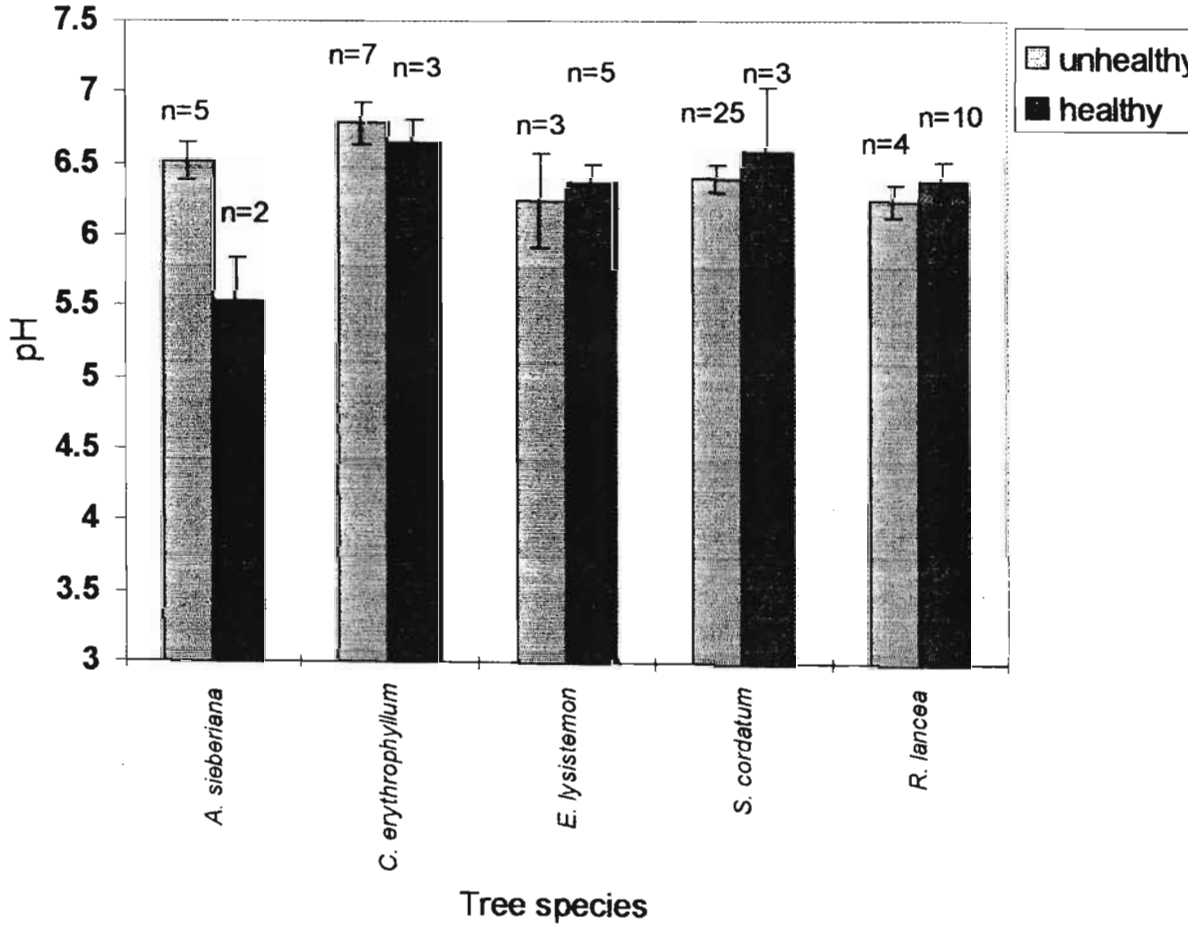


Figure 3.4: The relationship between soil pH and species health in August 1996. Results are mean values with standard errors.

The mean percentage soil stone content on the stability berm was 39.95% (Std error 1.18%). The highest stone content measured was 77.1% and the lowest 19.1%. No significant difference ($p<0.05$) was found between the soil stone content for healthy and unhealthy trees for the analysis of all the tree species or the individual species (Figure 3.5). This showed that although the stone content of the soil was high, it did not appear to be a primary cause for the difference in health of the trees.

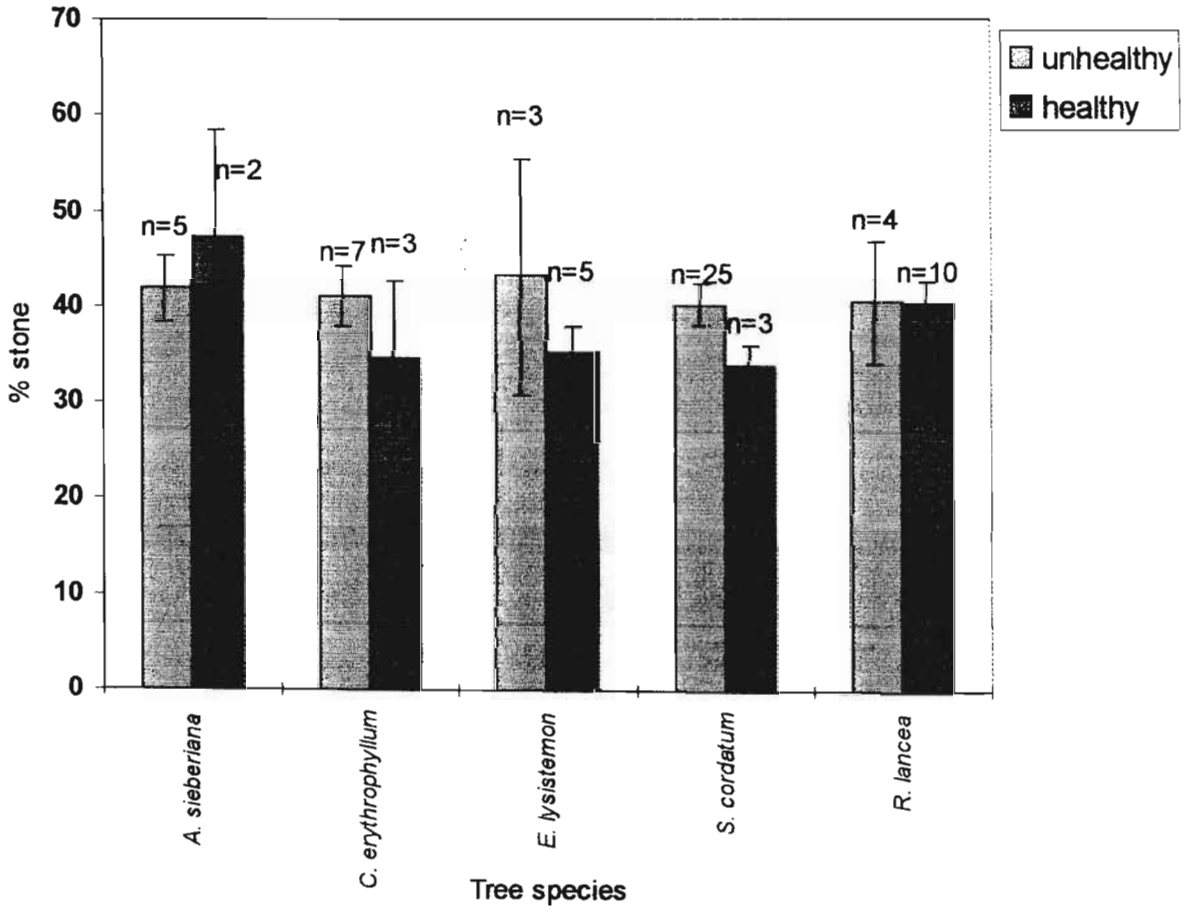


Figure 3.5: The relationship between % stone and the species health in August 1996. Results are mean values with standard errors.

The mean percentage soil moisture on the stability berm was 14.75 % (Std error 0.19) with a minimum of 4.5% and a maximum of 23.4%. Figure 3.6 shows the different soil moisture contents found in relation to healthy and unhealthy trees. *Acacia sieberiana*, *Combretum*

erythrophyllum and *Rhus lancea* had no significant ($p<0.05$) difference in soil moisture between healthy and unhealthy plants. However, the unhealthy trees of *Erythrina lysistemon* and *Syzygium cordatum* were exposed to a significantly ($p<0.05$) lower soil moisture content by comparison to the healthy trees. This could possibly suggest that the poor health of some individuals of *Erythrina lysistemon* and *Syzygium cordatum* may be due to soil moisture conditions.

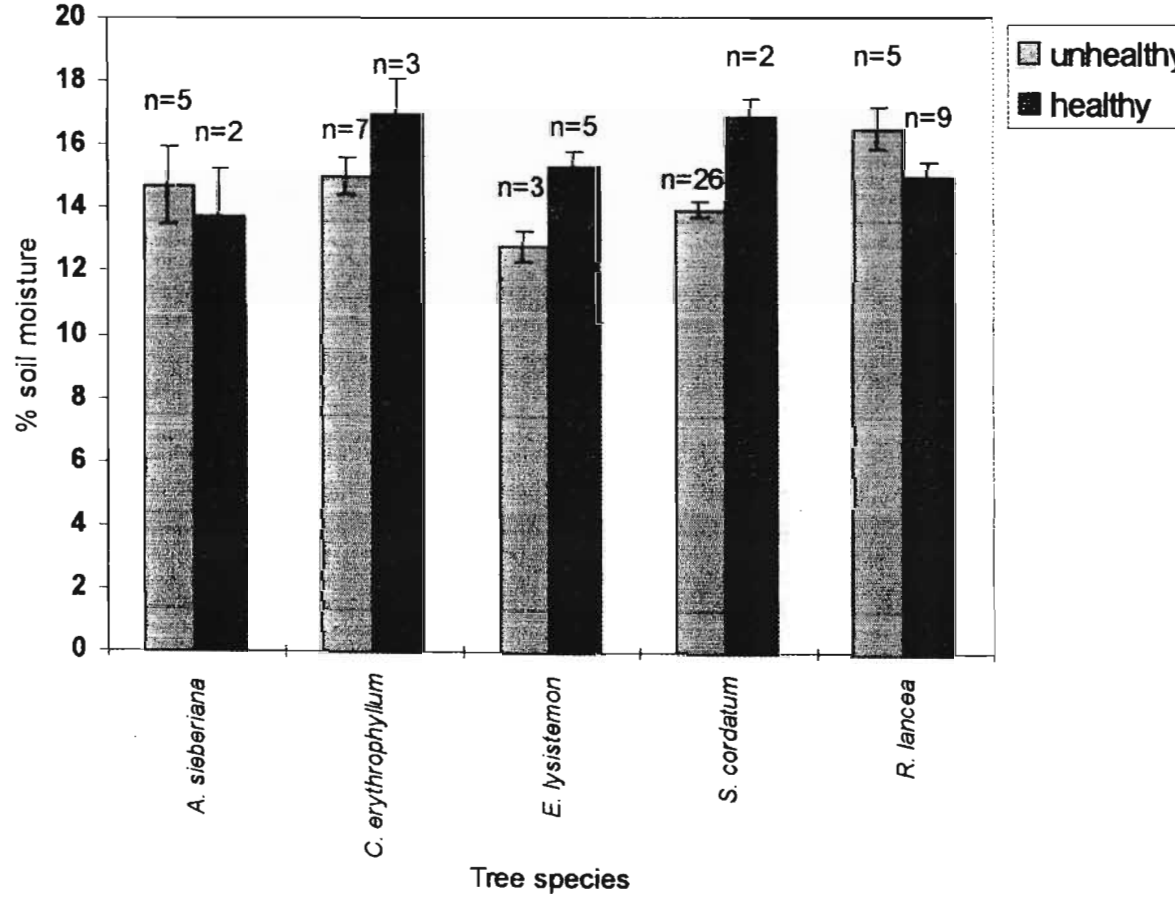


Figure 3.6: Relationship between soil moisture and species health in August 1996. Results are mean values with standard errors.

3.4 DISCUSSION

Taking into account that the landfill site is still fully operational and only a section was being revegetated, the major cause of tree mortality was due to disturbance by earth moving machinery. This point illustrates that over and above consideration for the harsh environmental conditions on landfill sites it is important to have careful planning and management of areas that are being revegetated in order for successful vegetation establishment to be achieved.

Unfortunately, for many of the species planted the replication was too small for conclusions to be made, therefore, only the performance of the following species could be assessed in greater detail: *Acacia sieberiana, Combretum erythrophyllum, Erythrina lysistemon, Syzygium cordatum* and *Rhus lancea*. However, although the numbers of individuals limited the interpretation of the data, *Strelitzia nicolai, Harpephyllum caffrum, Acacia xanthophloea* and *Hibiscus tiliaceus* appeared to be the relatively more healthy species out of those which had less than five individuals (Table 3.3).

Seasonal variation in deciduous trees effected the health ranking system (Table 3.4). *Erythrina lysistemon* was a good example of how a deciduous species which previously (May 1996) appeared 'unhealthy' became considerably more 'healthy' later in the year (August 1996). This emphasises the need for a less subjective and more absolute measure of plant health. It also highlighted the need for long term observation through all of the seasons, especially for deciduous species, in order to get a more accurate interpretation of the species performance. Unfortunately many of the trees were destroyed in October making the longer term monitoring of the trees on the stability berm impossible.

In terms of the effect of the environmental variables measured on the health of the trees, it would appear that soil pH, stone content and % moisture had little influence on the trees. Very little variation in pH throughout the area of investigation was measured. The soil pH on the stability berm was within the normal range of pH 4 – 8 for landfill restoration (McKendry, 1996; Moffat & Bending, 1992).

The mean stone content of the soil, of 40% (±1.2%), greatly exceeded the soil specification standards for landfill restoration of <10% (2-50mm) by dry weight (McKendry, 1996). However, in the comparative study here, the stone content of the soil did not affect the health of the trees. Stone content probably only represents an important factor when within a small size range (2-25mm), where it can prevent seed germination and root development (Mc Kendry, 1996). Although the stability berm cover material consists of a high percentage of stones within a small size range (2-25mm), the trees were several years old and had developed roots when planted, and so would not be as easily affected.

Syzygium cordatum and *Erythrina lysistemon* were the only two species that showed any significant difference between the two health categories for soil moisture (Figure 3.6). *Syzygium cordatum* is naturally always near water and often forms stands in pure swamp forest (Palgrave, 1984), therefore it is very likely to be sensitive to moisture conditions and will find low moisture levels challenging. *Erythrina lysistemon* is found in a much wider range of habitats from dry woodland to coastal dunes but usually in high rainfall areas (Palgrave, 1984). However, the unhealthy specimens of *Erythrina lysistemon* were exposed to lower soil moisture conditions in comparison to the other tree species (Figure 3.6), possibly providing an explanation for the significant difference in health for soil moisture. The general pattern of lower soil moisture for unhealthy plants (Figure 3.6), although not

significant, may indicate that moisture could present a problem to tree health during the months with lower rainfall.

High methane concentrations were closely associated with the poor health of the trees on the stability berm, as found in many other investigations (Chan *et al* 1991; Flower *et al*, 1981; Flower *et al*, 1977; Spreull & Cullum, 1987). This suggested that landfill gas infiltration into the soil atmosphere in the root zone was the key environmental variable measured influencing tree health. Methane is not directly toxic but is a good indicator of the presence of other toxic landfill gas components (Chan *et al* 1991). The high methane concentrations usually indicates anaerobic soil conditions and the possible presence of toxic gases such as carbon dioxide, ethylene and hydrogen sulphide which are often responsible for poor tree health (Leone *et al* 1977, Dobson & Moffat, 1994).

Soil carbon dioxide levels increased in a linear manner with soil methane concentrations confirming the findings of Chan *et al* (1991) and Lan and Wong, (1994). This indicated that the high carbon dioxide levels were also associated with the poor health of the trees. Carbon dioxide is an important component of landfill gas as it is toxic to plants in high concentrations (Arthur, *et al* 1981; Barry *et al* 1987; Chan *et al* 1991; Flower *et al* 1981; Leone *et al* 1977). The twenty two percent carbon dioxide concentration measured in high gas areas of the stability berm was higher than the range of 15-20%, which is lethal to most plants (Chan *et al* 1991; Chang & Loomis, 1945). Therefore, carbon dioxide levels were likely to be responsible for a large proportion of the trees poor health, however, low oxygen and trace gases such as ethylene and hydrogen sulphide may also have contributed to poor tree health.

The entire stability berm was characterised by patches of high and low methane and carbon dioxide concentrations, indicating a large spatial variation in landfill gas, commonly found on landfills (Wong *et al* 1992). Unlike that found by Lombard and Associates (1994), no variations in landfill gas concentrations in relation to atmospheric conditions were measured, however, the range of atmospheric temperature and pressure causing variations in landfill gas levels were not reported by Lombard and Associates (1994). A possible explanation for the lack of variation in landfill gas concentrations with climatic conditions, in this investigation, may be the small variation in these conditions experienced during the survey period.

The tree species were found to respond differently to the methane concentrations possibly indicating differential tolerance to landfill gases. *Acacia sieberiana* and *Syzygium cordatum* were found to have little or no tolerance with no healthy plants found exposed to methane. *Combretum erythrophyllum* had healthy trees surviving in very low methane concentrations. *Rhus lancea* had healthy trees surviving at a relatively higher mean methane concentration of approximately 9%. However, the species which showed the most tolerance to landfill gas was *Erythrina lysistemon*, which showed no significant difference between healthy and unhealthy species even though it was exposed to the highest methane concentrations of approximately 34% (Figure 3.3).

This investigation provided insight into the problems and challenges associated with revegetation of a landfill. It isolated two key factors associated with tree death, namely human disturbance, which refers to the unforeseen earth moving activity, and landfill gas. Better management and control can remove human disturbance, however, the removal of landfill gas is expensive, and not entirely successful, therefore, the search for tolerant

species is of significance. The species planted on the site provided preliminary data suggesting that there was a range of tolerance within indigenous tree species, which would be worthwhile investigating further.

CHAPTER 4: TREE GROWTH AND SURVIVAL: A FIELD EXPERIMENT

4.1 INTRODUCTION

The benefits of encouraging vegetation growth on operational and complete landfills has been well documented (Dobson & Moffat, 1994; Erickson *et al* 1994; Ettala *et al* 1988; Menser *et al* 1979). Trees have an especially important role, in terms of aesthetics, when reclaiming completed sites for parks, golf courses, and other similar amenities as well as for the screening of operational sites (Dobson & Moffat, 1994; Flower *et al* 1981). However, there are many factors limiting plant growth, especially trees, on landfills (Chan *et al* 1991; Lan & Wong, 1994; Dobson & Moffat, 1994; Ettala *et al* 1988; Flower *et al* 1981; Gill, 1970; Gilman *et al* 1981; Insley & Carnell, 1982; Leone *et al* 1983; Leone *et al* 1977; Moffat & Houston 1991). The amelioration of these factors can be very expensive and often less than completely successful. Therefore, the use of tree species tolerant to landfill conditions, when possible, can be of great benefit for revegetation success (Flower *et al*, 1981; Robinson *et al* 1992). The present study investigated the relative tolerances of indigenous tree species to the landfill environment with a special emphasis on landfill gas.

Using the results from the preliminary investigation (Chapter 3), ten indigenous tree species were selected for a more rigorous and on-site field study. The experimental screening of species in the field prevents the elimination of minor, or unforeseen detrimental environmental conditions. These may individually or in combination limit tree growth and survival. The hoped for outcome being the selection of species that are tolerant to the landfill environment as a whole and not just particular, individual components. In summary, the experiment has an element of a bioassay approach together with the measurement of certain variables to investigate the reasons for any differences in tree

performance. These measured variables included concentrations of gases (methane, carbon dioxide, oxygen) and the temperature of the soil in the root zone, and basic soil physical and chemical characteristics. This provided for some insight into the reasons for poor tree growth and thus a focus for amelioration procedures to overcome the potentially limiting environmental factors, and so facilitate successful tree establishment on landfills.

4.2 MATERIALS AND METHODS

4.2.1 Species selection

Nine tree species from the preliminary investigation were selected using two criteria: firstly, that they were readily available from commercial retailers in numbers greater than 70; and secondly, that they were the most successful in terms of survival in the preliminary investigation on the main stability berm. The majority of the species which survived best on the stability berm tended to be those found naturally growing in potentially waterlogged habitats (Palgrave, 1984; Pooley, 1994). To investigate further this assumption *Barringtonia racemosa,* a commercially available tree species which is characteristically found in swamp forest communities (Palgrave, 1984; Pooley, 1994) was added to the list of species to be screened. Therefore, the following ten experimental species were chosen: *Acacia sieberiana; Acacia xanthophloea; Barringtonia racemosa; Combretum erythrophyllum; Hibiscus tiliaceus; Erythrina lysistemon; Harpephyllum caffrum; Rhus lancea; Strelitzia nicolai* and *Syzygium cordatum.*

4.2.2 Experimental design

Considering that landfill gas in the soil was a key environmental condition related to poor tree health, the presence of landfill gas in the area to be used in the field experiment was

essential. In the preliminary investigation (Chapter 3) the lack of homogeneity of landfill gas concentrations (measured as methane concentrations) in the soil made the assessment of particular species performance in relation to landfill gas concentrations difficult. Therefore, for the field experiment it was important that an area of the landfill which was relatively homogenous in terms of landfill gas concentrations in the soil was used. An area of the landfill was investigated for its suitability for the field experiment (Figure 1.2). This area was temporarily complete and had approximately 30m of waste underneath it, which had been in-filled since 1980, and then covered with approximately 0.5m of waste soil. This area was beyond the effective range of the recently installed gas reclamation wells (Dorkin, D. 1996 *pers comm*), thus ensuring a negligible effect of active gas removal on the concentrations of landfill gas in the soil.

A 50m by 50m section of the area was then selected for its relatively flat topography and homogeneous appearance in terms of soil structure and moisture (Figure 1.2). Within this 50m by 50m section 13 gas samplers, with the same design as those used in the preliminary investigation (Chapter 3), were installed in a grid pattern. Methane concentrations in the soil were measured once a week for three weeks (Table 4.1). Table 4.1 shows that the spatial and temporal variation in methane concentrations during the 3-week period of monitoring was acceptably low. The over-all mean methane concentration for the plot was 52 % which was considerably higher than the mean value of 14% measured on the stability berm in the preliminary investigation. Although the gas concentrations were considerably higher, the plot was regarded as suitable for the field experiment. There was very little variation spatially or temporarily for 10 of the sampling points in the plot. However, three areas of the plot did have lower methane in the soil, as indicated by the measurements from gas samplers 8, 10 and 13 (Table 4.1), indicating a

slight variability in gas emissions within the plot. It was, therefore, decided that a replicated grouped experimental design (see below) would be the best for the planting of the trees, so as to account for this apparent heterogeneity where pockets of lower concentrations may be found.

This 50m by 50m area was completely fenced so as to prevent any accidental damage to trees by vehicles during the field experiment. Within the fenced area two 25m by 25m experimental plots were established. One received 1m of topsoil (the topsoil plot) whilst the second plot received no topsoil and had only the original 0.5m deep waste soil cover material. A control plot was situated off the landfill approximately 1000m away from the experimental plots, in the Randles Road Municipal Nursery (Figure 1.2). The topsoil used in the experiment was loose tipped into position using a back actor excavator. Five gas samplers which were installed on this control plot detected no methane during a monitoring period of 3 weeks (4-18th November 1996). The underlying substrate of the control plot was yellow clay resulting from the extensive weathering of a dolerite intrusion. On top of soil present at the control site a 1m layer of topsoil, from the same well-mixed stockpile as used on the first plot on the landfill (the topsoil plot), was also placed.

In each of the 3 plots the trees were planted in seven replicated groups. Each of the seven groups had one replicate tree of each species, planted randomly at 1.5m centres. The grouped planting of species was regarded as a more satisfactory way of accounting for site and substrate heterogeneity than a strictly random design for the whole plot. In particular, it was possible that there were pockets of higher or lower landfill gas concentrations in the plots on the landfill (Table 4.1).

Table 4.1: Methane concentrations measured at 13 sample points in the soil within a 50m by 50m area of the landfill between the 4th and the 18th of November 1996, in assessment of its suitability for a field experiment

	% methane		
Gas sampler	Week1	Week 2	Week 3
1	65	65	66
2	60	58	60
3	60	60	58
4	55	55	55
5	55	55	55
6	55	55	55
7	55	55	55
8	55	30	15
9	56	56	55
10	32	35	30
11	55	55	55
12	51	65	60
13	35	40	35
Mean % methane (Std. Error)	53 (3)	53 (3)	50 (4)

The trees were obtained in January 1997 from Randles Road Municipal Nursery in 6*l* potting bags. Individual plants of each species were selected so as to ensure they were approximately the same age and size (2 years old). The potting bags were cut off and the tree roots were then slightly loosened and planted with the attached potting soil directly into the ground of each plot. After planting they were provided with water on a daily basis for the first 4 weeks only. Aftercare of the trees involved the regular weeding of the plots to prevent competition from naturally established grasses and forbs.

4.2.3 Tree performance

Stem diameter and height growth, survival, leaf chlorophyll fluorescence, general health appearance, total aboveground biomass, total leaf area and rooting depth were used to determine treatment effects and differential species response.

The stem diameter and the height of the trees was measured when they were first planted (20/1/97), after 7 months (20/8/97) and finally after 14 months (20/4/98). Data was expressed as the growth increment between these dates. Stem diameter was measured 50mm from the base of the stem using electronic digital callipers. To avoid inaccuracy due to the non-symmetrical shape of stems the orientation of the diameter measurement was taken consistently along a north -south axis. The tree height was measured from the base of the stem to the apical shoot using a steel tape.

The general appearance of the individual trees was also monitored as an assessment of the tree health. The trees were observed and put into one of five categories according to their overall appearance which provided a ranking system from 'dead' to 'very healthy', as used for the trees in the preliminary investigation (Table 3.1). Although this system intrinsically was subjective, the health rankings were all completed by the same person so as to help remove bias in the results. The number of trees of each species that were still alive within each treatment at the end of the experiment provided the measurement of survival.

The above ground biomass was calculated by adding the dry mass of the stem and leaves of each of the trees after the 14 month experimental period. Due to the size and number of trees the dry mass of the stem and leaves was calculated from the fresh weight by drying a sample from each species from each of the experimental plots and calculating a fresh

weight to dry weight correction factor. Although the trees were roughly the same age and size when they were originally planted, the final mass of the trees was expressed as a ratio of the original height of the tree (relative biomass) in order to standardise the data. The total leaf area of each tree was calculated by determining the ratio of leaf mass to leaf area of a sample of leaves and then using this value to estimate the total leaf area from the total leaf mass for each tree.

In order to describe the root morphology of the trees on the control and landfill plots a profile wall trench was excavated for each species on each plot (Total n=30). Using a back actor excavator a 1m deep trench was excavated 300mm from the base of the stem of each tree. The profile wall was levelled with a straight edge and the protruding roots were trimmed. A 100cm X 90cm steel grid, divided into 10cm square blocks was placed onto the profile wall and the roots within the <5mm, 5-10mm, >10mm size classes, within each block were recorded. However, in practice there was a very small range in root diameters seen in the profile walls with 99.5% of the roots less than 5mm in diameter. Therefore the size classes were not used and overall root density with depth was assessed.

4.2.4 Soil gases and soil temperature

Seven gas probes, of the same design to those used in the preliminary investigation (Figure 3.1), were inserted into the substrate in each of the three plots, one within each replicated group of trees. Gas samples were monitored on a monthly basis for percentage methane, carbon dioxide and oxygen in air with a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. Thus monitoring the gas concentrations in the soil surrounding the roots of the trees in each experimental plot. The variation in mean gas concentrations measured once a month was statistically analysed for significance. The mean atmospheric pressure and

mean daily temperature on the days of gas monitoring were compared with mean percentage gas measured. This was carried out to determine if meteorological conditions effected the gas concentrations in the root zone. The atmospheric pressure and temperature were measured by the South African Weather Bureau at Durban International Airport, approximately 20km south of the landfill.

Soil temperatures 30cm below the surface were taken using a Sharp YFE YF-1062 digital thermometer and compared with ambient air temperature. This was done by inserting the digital thermometer into each gas probe on the three plots.

Further gas measurements were made to investigate the relationship between concentration and depth. Methane, carbon dioxide and oxygen concentrations were measured at 3 soil depths at 4 different points on control plot and landfill plots. This was done by placing 3 different lengths of gas samplers within a $0.25m^2$ area at each of the 4 sampling points on the plots. Thus, gas was sampled from three depth intervals, namely 10-20cm, 25-30cm and 40-50cm. Methane, carbon dioxide and oxygen concentrations were measured using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser on 4 separate days. The relationship between the gases measured and the soil depth was analysed using regression analysis in order to determine the equation of best fit for the data.

4.2.5 Soil chemical analysis

Each plot was divided equally by area into four sub-plots, from each sub-plot three soil samples were taken at random and pooled together. The soil samples from each plot were taken at a depth of 5-10cm sealed into plastic bags and thoroughly mixed. From these samples, sub-samples were taken for soil analyses. Four sub-samples from each plot were

sent to the KwaZulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: extractable P ; K ; Ca ; Mg ; Zn ; Mn ; Extractable Acidity; Total cation; Acid saturation; pH (KCl); organic carbon percentage; and percentage clay (Hunter, 1974). This gave a basic set of soil variables for the comparison between the experimental plots.

Soils were air dried, large stones removed, and then lightly ground to breaks soil clods and sieved through a 1mm sieve. The sample density of each sample was calculated by taking a known volume of soil and determining its mass. The sample density was used to determine the mass of soil used for each test, which was carried out on a volume basis. The measured concentrations of each soil constituent were converted using the sample density from mg/ℓ to mg/kg. The pH of the soil was determined using a pH electrode placed in a $10cm^3$ soil: $25cm^3$ 1M KCl suspension which was mixed and allowed to stand for 60 minutes.

Extractable calcium, magnesium and acidity were determined from $2.5cm^3$ soil: $25cm^3$ 1M KCl solution which was stirred for 10 minutes and then filtered through Whatman No. 1 filter paper. The reagent used for Ca and Mg determination was a strontium solution consisting of 380g $SrCl_2.6H_2O$ added to 2 litres of concentrated HCl and made up to 40 litres with de- ionized water. A $5cm^3$ aliquot of the KCl soil filtrate was diluted five times to $25cm^3$ and added to $20cm^3$ of the strontium solution, this was then used to determine Ca and Mg by atomic absorption with the following instrument settings: Ca was determined at 422.7nm, current of 3.7 mA and a slit width of 0.5nm; Mg was determined at a wavelength of 589.6nm, current of 3.5mA and slit width of 0.5nm. The reagents used for extractable acidity determination from the KCl soil extract included a solution of phenolphthalein. This was made up by adding 5g phenolphthalein powder into $500cm^3$ ethanol and adding

approximately 500cm^3 water to make up a 1 litre stock solution, a diluted phenolphthalein solution was then made by adding 300cm^3 of the stock to 10ℓ of de-ionized water. A 10cm^3 aliquot of the KCl soil extract was diluted two times to 20 cm^3 and added to 10cm^3 of de-ionized water containing 2-4 drops of the diluted phenolphthalein solution. This was titrated with 0.005M NaOH to determine the centimoles of acidity per litre of soil using the following equation:

$$\frac{\text{No. cm}^3\text{ 0.005M NaOH - No. cm}^3\text{ reagent blank}}{2} = \text{centimole of acidity per litre of soil (cmol(+)/l)}$$

Extractable phosphorus, potassium, zinc and manganese was determined using an extracting solution prepared by dissolving 197.6g NH_4HCO_3 in de-ionized water, dissolving 37.2g disodium salt of ethylenediaminetetra-acetic acid (EDTA) in de-ionized water, dissolving 3.7g NH_4F in de-ionized water, and measuring out 100cm^3 of concentrated solution of Superfloc (grade N100) consisting of 10g of the flocculant in 2000cm^3 of water. The above mentioned solutions were mixed into 5ℓ of distilled water and brought to a final volume of 10ℓ. The pH of the prepared ammonium bicarbonate extracting solution was then adjusted to 8 using a strong ammonia solution.

The phosphate colour reagent was prepared by placing 2g antimony potassium tartrate in 800cm^3 distilled water and mixing with 300cm^3 of concentrated H_2SO_4 and allowed to cool overnight. 15g of ammonium molybdate was dissolved in 600cm^3 of water and added to the acid antimony potassium tartrate solution and brought to a volume of 1ℓ using distilled water. On the day of use 150cm^3 of the molybdate solution was diluted to 1ℓ with a

solution containing 1g gelatine per litre of warm water, and 1g of ascorbic acid was added and mixed. Phosphate standards were made by dissolving 0.4390g KH_2PO_4 in 975cm^3 de-ionized water, and adding 25cm^3 7N H_2SO_4. This provided a stock solution containing 100 mgℓ^{-1} P. From the P stock solution 0, 10, 20, 40 and 60cm^3 were taken and made up to 1 litre with the ammonium bicarbonate extracting solution. This provided phosphate standards of 0, 1,2,4,6 mgℓ^{-1} P.

The potassium standards were made by taking the stock and making it up to 1ℓ, thus a concentration of 600 mgℓ^{-1}. Zero, 10, 20, 50 and 100cm^3 of the K solution were made up to 1ℓ using the ammonium bicarbonate extracting solution. This provided potassium standards of 0, 6.1, 12.2, 31.6 and 66.7 mgℓ^{-1}

Zinc and manganese standards were made up by taking 50cm^3 of 1000 mgℓ^{-1} Zn and Mn atomic absorption standards and adding to 9950cm^3 distilled water, thus a concentration of 50 mgℓ^{-1}. Zero, 2,4,10, and 20cm^3 of the Zn and Mn 50 mgℓ^{-1} stock solution was made up to one litre with ammonium bicarbonate extracting solution. This provided zinc and manganese standards of 0, 0.1, 0.2, 0.5, and 1 mgℓ^{-1}.

The aforementioned set of reagents were used for determining extractable P, K, Zn and Mn with the following procedures. A 2.5cm^3 scoop of each soil sample was shaken with 25cm^3 of ammonium bicarbonate solution for 10 minutes. They were then filtered through Whatman No. 1 filter paper and kept at a constant temperature of 22°C. Extractable P was determined by taking a 2cm^3 aliquot of the filtrate, adding 8cm^3 distilled water and 10cm^3 of ammonium molybdate colour reagent. The same dilution was added to the P standards

and after 40 minutes the absorbance values at 670nm with a spectrophotometer were measured. Extractable K was determined by taking $5cm^3$ of the ammonium bicarbonate soil filtrate and adding $20cm^3$ of de-ionized water. The same dilution was added to the potassium setting standards and K was determined by atomic absorption with the following settings: wave length(λ)=766.5nm; current= 5,0mA; slit width=1.0nm. Extractable Zn and Mn were determined on the remaining undiluted ammonium bicarbonate soil filtrate with the following atomic absorption settings: Zn: wave length= 213.9nm ; Mn wave length= 279.5nm and for both Zn and Mn a Current= 5.0mA ; Slit width= 1.0nm.

The percentage organic carbon and percentage clay content of air-dried soil samples was determined by absorbance of light in the infrared region of the spectrum. Nineteen different wavelengths in the near infrared region of the spectrum were used to scan the soil samples and the absorbances were recorded on computer. The absorbances were then used in a set of formulas used to calculate organic carbon and clay percentages. The formulas were obtained by scanning a range of soils that had been analysed using standard wet chemistry methods for % carbon and % clay determination. A multiple linear regression analysis was performed to establish the relationship between the relevant soil constituent and the absorbances of the wavelengths best suited to analyse a particular constituent.

Using the University of Natal facilities sub samples of soil were also analysed using X-ray fluorescence spectrometry for total Si; Al; Fe; Mn; Mg; Ca; Na; K; Ti; P; Nb; Y; Rb; Zr; Sr; U; Th; Zn; Cu; Ni; Cr; V; La; Ba; Sc; S; Cd; Pb; Ga; Co; Ce; Nd; As. The samples were milled to less than 40 μm particle size. After mixing the residue with 5.0 g lithium metaborate and 25 mg lithium bromide, it was fused at 1200 °C for 20 min. The resultant

samples were analysed by wavelength dispersive x-ray fluorescence spectrometry using a Phillips PW1480 spectrometer.

Three, approximately 50g, samples of air-dried, sieved soil from each plot was saturated with de-ionized water and allowed to stand for 24 hours. The samples were then centrifuged using a Beckman G.P. centrifuge (No. 355953) at 3700rpm (relative centrifugal field = 2127.4) for 30 minutes to extract the supernatant. (Jackson, 1962). The conductivity of the supernatant was measured using a Crison MicroCM 2201 conductivity meter corrected to 25°C.

4.2.6 Soil physical analysis

The mean soil moisture content of each plot was calculated by loss of weight after oven drying at 105°C and expressed as a percentage. This was done using six fresh 10g sub-samples of soil from each plot. (Grimshaw, 1989). The remaining soil from these samples was air-dried. It was then lightly ground using a mortar and pestle so as to break up the clods and then sieved through a 2mm sieve. The weight of stones removed by sieving in relation to the original weight of air dried soil was expressed as the percentage stone content of the soil sample (Grimshaw, 1989).

4.2.7 Data analysis

Statistical analysis of the data was completed using Statgraphics Plus Statistical Graphics System, version 7:0, computer software produced by Manugistics, Inc. and Statistical Graphic Corporation. Data were analysed using an analysis of variance. If there was a significant difference ($p<0.05$) in data sets with more than two sample variables then

Scheffe multiple range test was performed by constructing intervals for pair-wise differences of means to determine which differences were significant ($p<0.05$). However, this was only done if the residuals of the data were normally distributed, as tested using a Kolmogorov-Smirnoff test for normality, ($p>0.05$). If the data were not normally distributed, and transformations were unsuccessful, a Kruskall-Wallis analysis for non-parametric data was used. If a significant difference ($p<0.05$) was found using the Kruskall- Wallis analysis then a Mann-Witney U test was used to analyse the data in a pair-wise manner so as to determine which differences were significant ($p<0.05$) (Zar, 1984). The relationship between two variables was evaluated also using a scatter plot and Pearson's Product-moment correlation analysis and regression analysis (Zar, 1984).

4.3 RESULTS

4.3.1 Soil gases

No significant change ($p<0.05$) in methane, carbon dioxide and oxygen concentrations in root zone, for all the plots, individual plots or individual gas samplers was found in relation to atmospheric pressure, daily temperature, or month. However, there was very little variation in the temperature and pressure between the different days on which gas measurements were taken. The maximum and minimum atmospheric pressure recorded for the days on which gas measurements were made were 1026.9 Mpa and 1010.9 Mpa respectively, with a mean value of 1016.2 Mpa (Std. error ± 1.19). The maximum and minimum temperatures for the days on which gas measurements were made were 25.8°C and 16.2 °C respectively, with a mean value of 21.0°C (Std. error ± 0.94). Therefore, it can be concluded that the temperature and pressure ranges experienced during the experiment did not account for the observed changes in methane, carbon dioxide or oxygen soil concentrations.

Figure 4.1 shows the carbon dioxide, methane and oxygen concentrations found in the root zone (50cm soil depth) of the control and experimental plots, calculated from the measurements taken from all of the gas samplers on each plot throughout the experimental period (14 months). Carbon dioxide was found in the root zone of all of the plots, however it was significantly ($p<0.01$) higher in the plots on the landfill. Of the two plots on the landfill, the plot without topsoil had a significantly ($p<0.01$) higher carbon dioxide concentration (48.3%) than that with topsoil (25.6%) (Figure 4.1). The presence of methane was only found in the plots situated on the landfill, with significantly ($p<0.05$) lower concentrations on the topsoil plot (22.3%) in comparison to the plot without topsoil (41.9%). The concentration of oxygen within the control plot (16%) was significantly ($p<0.05$) higher than in the landfill topsoil plot (3.2%) and the landfill plot without topsoil (0.6%). However, no significant difference ($p>0.05$) in oxygen concentrations between the two landfill plots was found (Figure 4.1).

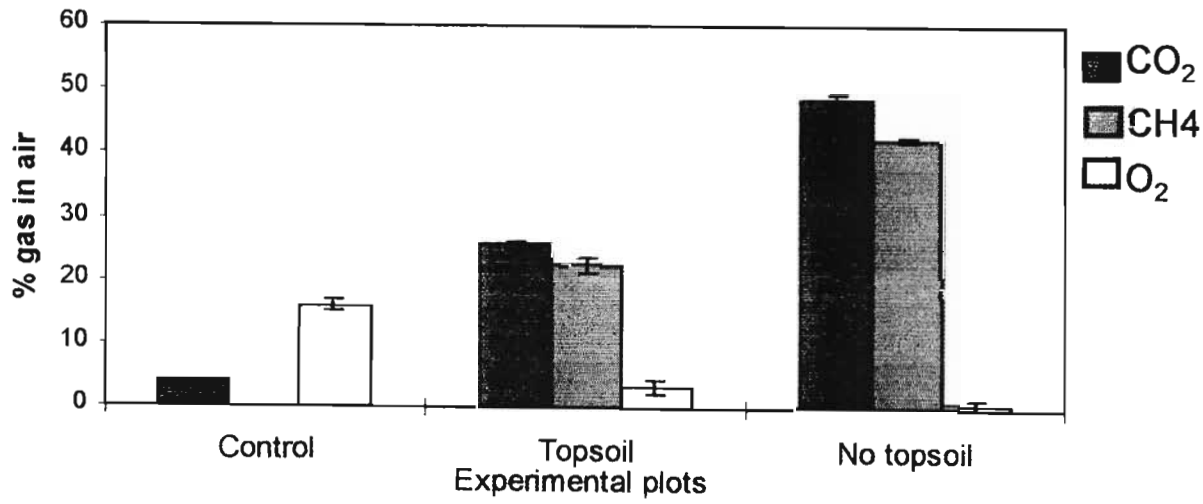


Figure 4.1: Carbon Dioxide, methane, and oxygen in the root zone of each plot measured on 14 occasions during the experiment. Results are mean values with standard errors

Carbon dioxide is found in relatively low concentrations (mean=3.9%) in the soil atmosphere of healthy aerobic soils, as shown in the control plot (Figure 4.1). The significantly higher concentrations of carbon dioxide and the presence of methane in the soil atmosphere of the experimental plots on the landfill, showed that the waste below these plots was having a significant effect on the composition and concentrations of gases in the soil atmosphere. The oxygen concentrations in the soil of the control plot were close to ambient air concentrations with low carbon dioxide and no methane. However, oxygen concentrations on the landfill plots were almost zero with high concentrations of carbon dioxide and methane. These results showed that anoxic soil conditions prevailed on the landfill and that it was related to the increased carbon dioxide and methane in the soil atmosphere.

By the comparison of the two plots on the landfill the application of topsoil was found to significantly ($p<0.01$) decrease the concentrations of carbon dioxide and methane found within the soil atmosphere (Figure 4.1). This difference in landfill gas concentrations was unlikely to be due to coincidental spatial variation in gas concentrations, as the area for the field experiment was found to be relatively homogenous in terms of landfill gas before the topsoil was applied (Table 4.1). The ratio of methane to carbon dioxide on the landfill plot with topsoil was 0.77 (Std error 0.06) and on the plot without topsoil was 0.87 (Std error 0.01). These ratios were not significantly ($p>0.05$) different, showing that although the volume of each gas in the topsoil layer was lower the relative composition of the gas had not changed. This is interesting as it suggests that the oxidation of methane into carbon dioxide was not the primary cause of lower methane in the topsoil plot (i.e. CO_2 levels, proportionally did not rise).

The relationships between CO_2, CH_4, and O_2 for all the individual gas measurements made on the control and the experimental plots are shown in Figures 4.2, 4.3 and 4.4. The landfill plot which received topsoil had a wide range of methane and carbon dioxide concentrations and this plot accounted for most of the variation in the whole data set. The data points along the y-axis of Figures 4.2 and 4.3 show that no methane was found in the control plot soil. Further regression and correlation analysis of the relationship between the gases measured in the landfill soil atmosphere was conducted by excluding the methane, carbon dioxide and oxygen data from the control plot from the statistical analyses (Table 4.2). However, to satisfy the assumption of normality of residuals for these tests, both the dependent and independent variables were transformed using an arcsine transformation where necessary. That being the proportions of each gas measured (P) expressed as the transformed value A (=arcsin $\sqrt{P}$). Conclusions from these results are made with reference to the Figures (4.2-4.4) and Table 4.2.

The carbon dioxide concentrations appeared to increase with increasing methane concentration (Figure 4.2). The methane and carbon dioxide data had a positive linear relationship ($y=0.56x + 8.8$, $R^2=0.73$, $p<0.01$) (Table 4.2). However, methane was only found in the soil atmosphere when carbon dioxide was in excess of 8.8%, as indicated by the y intercept of the regression analysis (Table 4.2).

Table 4.2: Linear Regression and Pearson's Product-Moment Correlation for the relationship between methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) in the soil atmosphere.

Relationship	Y intercept (transformed data)	y intercept [a] (Back transformed)	Slope	R^2	Correlation coefficient
[b] CH_4 (x) *versus* CO_2 (y)	0.30± 0.02	8.8%	0.56± 0.03	0.73	0.86*
[b] CH_4 (x) *versus* O_2 (y)	0.26± 0.02	6.6%	-0.27± 0.02	0.43	-0.66*
[c] CO_2 (x) up to 23% *versus* O_2 (y)		14.9%	-0.60± 0.1	0.54	-0.73*
[c] O_2 (x) *versus* CO_2 (y) up to 23%		20.7%	-0.89± 0.14	0.53	-0.73*

[a] [sin (arcsin transformed value x 180 ÷ PI)]^2 x 100
[b] These data were arcsin transformed
[c] The data were normally distributed, therefore there was no need for a arcsin transformation
* $p<0.01$

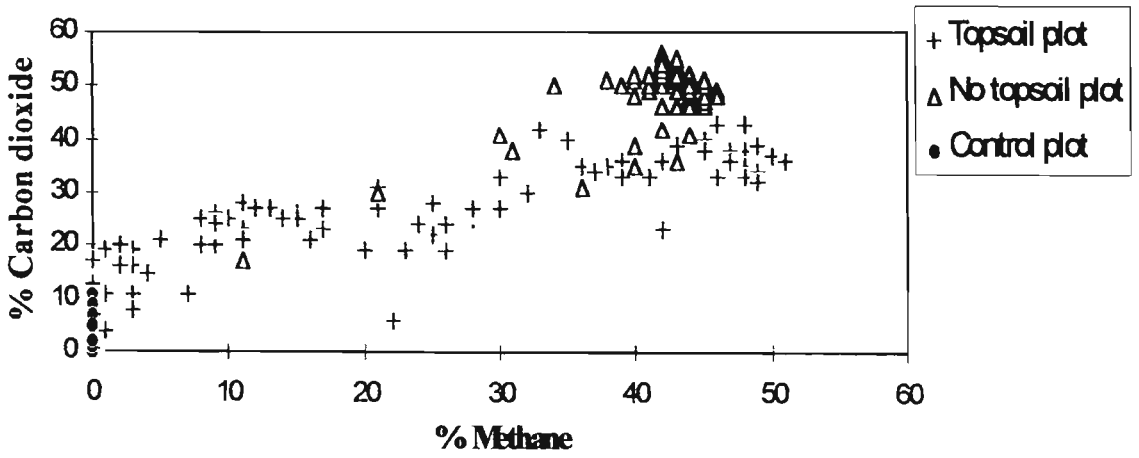


Figure 4.2: Relationship between methane and carbon dioxide in the root zone of the trees planted on the control and the experimental plots (Data points for each experimental plot given a different symbol).

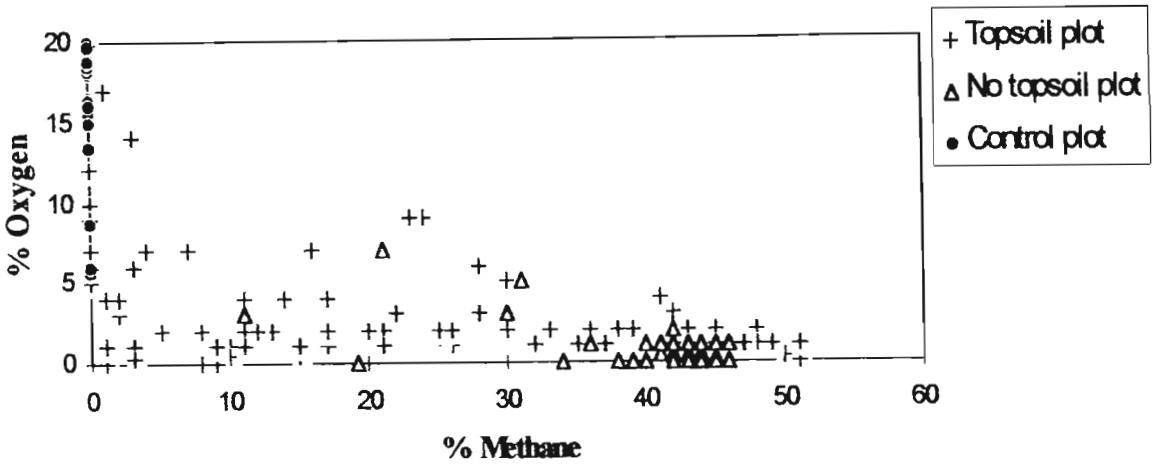


Figure 4.3: Relationship between methane and oxygen in the root zone of trees planted on the control and experimental plots (Data points for each experimental plot given a different symbol).

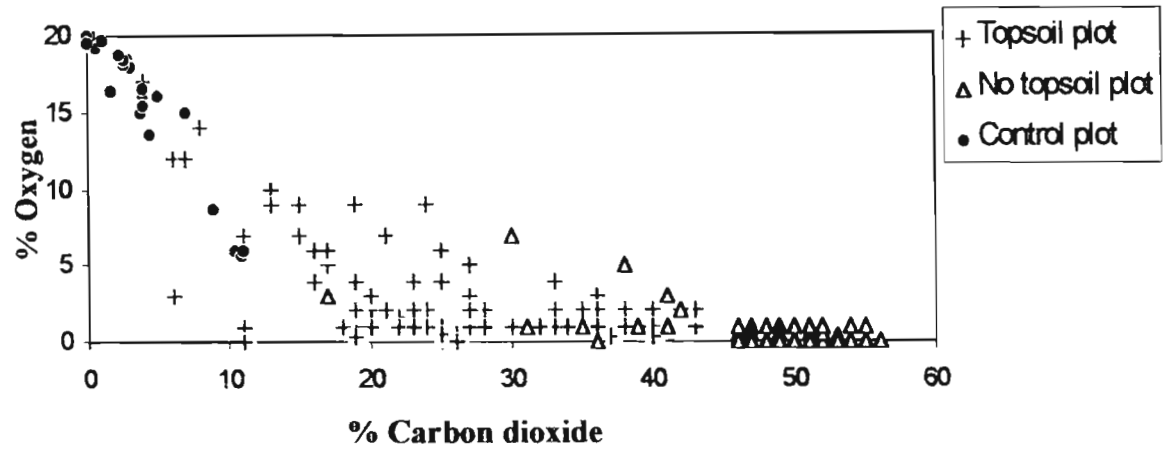


Figure 4.4: Relationship between the carbon dioxide and the oxygen in the root zone of the trees on the control and experimental plots (Data points for each experimental plot given a different symbol).

Methane and oxygen had a negative linear relationship ($y=-0.27x + 6.6$, $R^2=0.43$, $p<0.05$) (Table 4.2), showing that oxygen was lowered with increasing methane (Figure 4.3). The slope of the regression line was low (-0.27) indicating a very small change in oxygen with

increasing methane (Table 4.2). It was also noted that oxygen was already reduced to 6.6% before methane was detected, as indicated by the y intercept (Table 4.2).

The relationship between carbon dioxide and oxygen showed that oxygen concentrations were rapidly reduced, from ambient air concentrations to almost zero, as carbon dioxide increased to approximately 23% (Figure 4.4). Oxygen concentrations then remained close to zero and carbon dioxide concentrations continued to increase (Figure 4.4). A linear regression of the initial decline in oxygen, up to a carbon dioxide concentration of 23%, was calculated. A negative linear relationship (R^2=0.54) ($p<0.05$) with a very steep gradient (slope= -0.60) of decline was found (Table 4.2). This quantified the rapid depletion of oxygen, from ambient air concentrations. A further regression analysis with carbon dioxide as the dependant variable and oxygen as the independent variable showed that for these data oxygen was totally depleted at 20.7% carbon dioxide concentration (Table 4.2).

In summary, these results of individual gas measurements showed that methane was only detected in the soil when carbon dioxide concentrations were in excess of 8.8% and oxygen levels were already depleted below 6.6%. The ambient oxygen concentrations were reduced to zero when carbon dioxide had increased to 21%.

The results of the analysis of the soil gas composition at different soil depths within the control and experimental plots are shown in Figures 4.5; 4.6 and 4.7. As expected the concentration of methane and carbon dioxide increased and oxygen levels decreased with soil depth on the landfill experimental plots. A similar relationship was found on the control plot, however the gas concentrations measured were not as extreme and the lack of

underlying anaerobic waste decomposition resulted in no methane. The control plot trees had a maximum rooting depth of 70cm which coincided with an extrapolated oxygen concentration of 13% and carbon dioxide level of 3% (Figure 4.5).

The methane, carbon dioxide and oxygen concentration gradients in the landfill cover material were less steep than that found in the topsoil placed on the landfill. Thus there was higher methane and carbon dioxide and lower oxygen concentrations at shallower soil depths in the landfill cover material relative to the topsoil layer (Figures 4.6 and 4.7). This was probably due to relatively high compaction and poor soil structure of the landfill cover material, allowing for little atmospheric dilution of the landfill gas infiltration from depth. The shallower rooting depths on the landfill with or without a topsoil layer can be explained by the soil atmosphere conditions. On the landfill topsoil plot the maximum rooting depth of 40 cm coincided with a methane concentration of 53%, 20% carbon dioxide and 2% oxygen. On the landfill plot without topsoil, the maximum rooting depth of 20 cm coincided with a methane concentration of 57%, 27% carbon dioxide and 1% oxygen. Although the maximum rooting depths on the two landfill plots were different, it is interesting to note that soil gas composition at maximum rooting depth was reasonably similar. This suggests that the composition of the soil atmosphere was the key factor determining rooting depth.

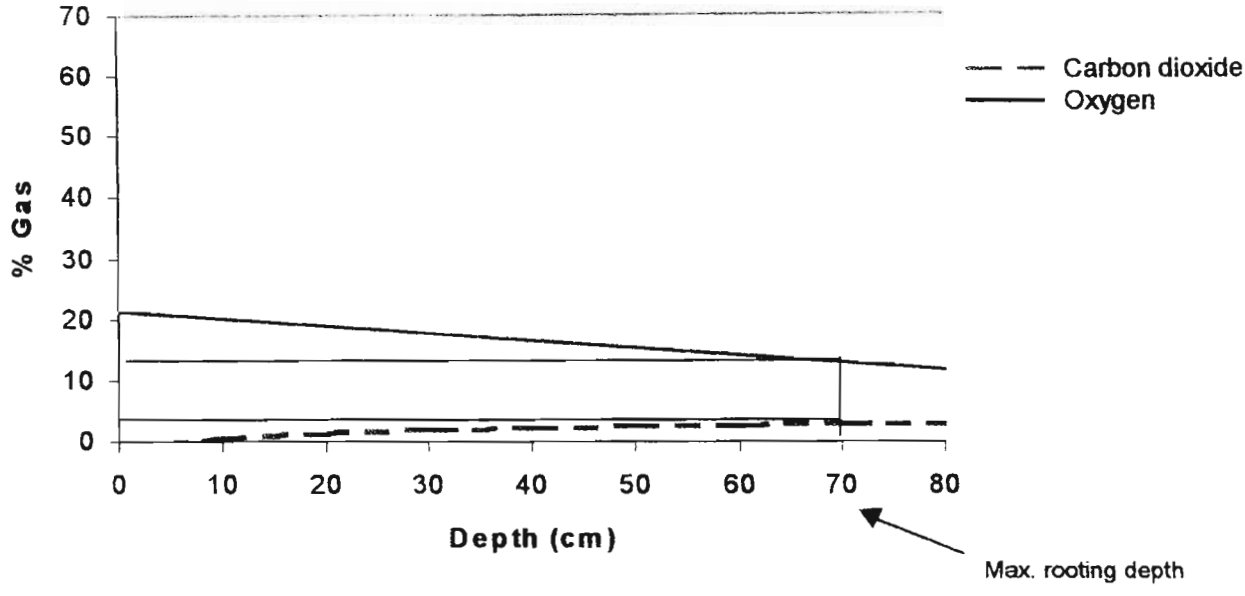


Figure 4.5: Regression models of gas concentrations measured with soil depth in the control plot topsoil layer. Carbon dioxide model equation: $y = 1.2078\ln(x) - 2.1747$, $R^2 = 0.94$, $p<0.05$. Oxygen model equation: $y = -0.1167x + 21.106$, $R^2 = 0.87$, $p<0.05$.

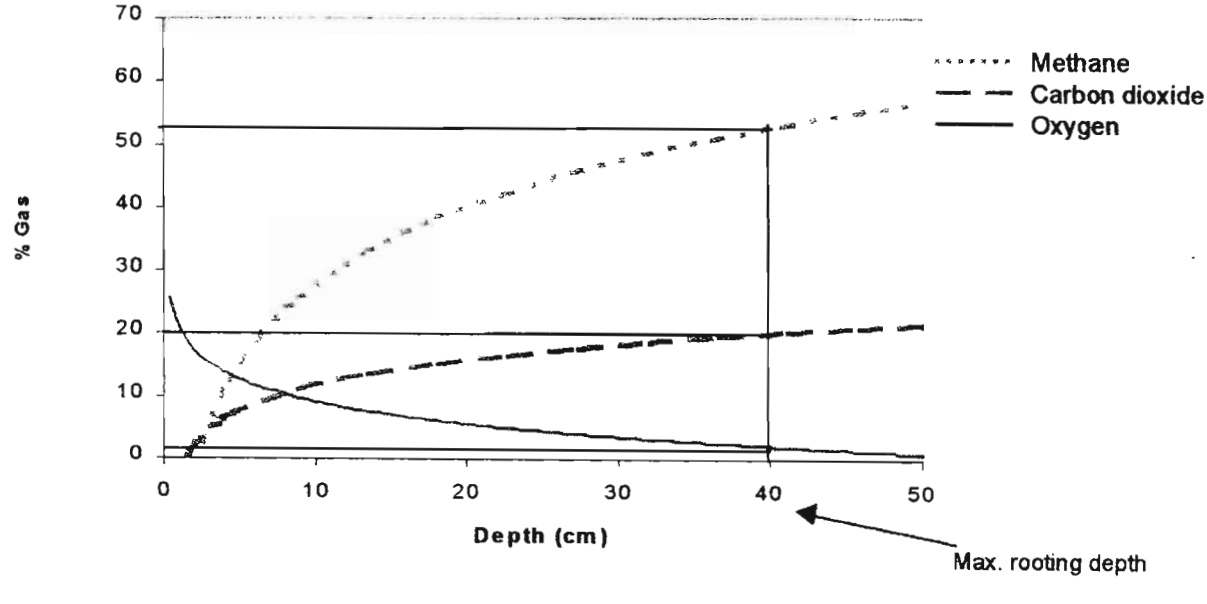


Figure 4.6: Regression models of gas concentrations measured with soil depth in the topsoil placed on the landfill (Topsoil plot). Methane model equation: $y = 17.906\ln(x) - 13.324$, $R^2=0.68$, $p<0.05$. Carbon dioxide model equation: $y = 6.0159\ln(x) - 1.9091$, $R^2=0.53$, $p<0.05$. Oxygen model equation: $y = -5.0934\ln(x) + 20.823$, $R^2 = 0.45$, $p<0.05$

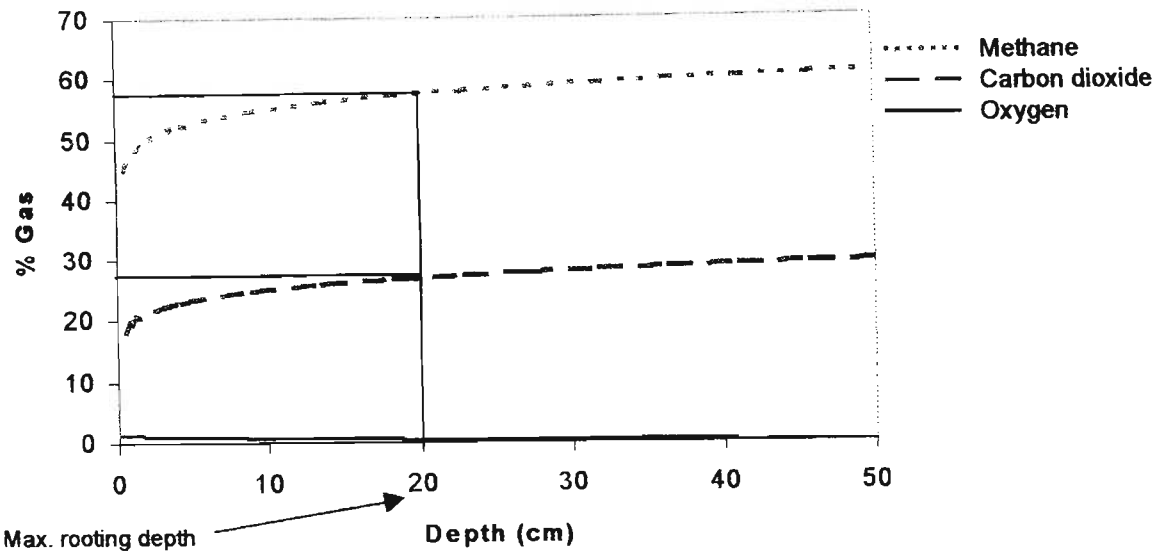


Figure 4.7: Regression models of gas concentrations measured with soil depth in the in the landfill cover material (No topsoil plot). Methane model equation: $y = 47.819\ x^{0.0617}$, $R^2 = 0.12$, $p < 0.05$. Carbon dioxide model equation: $y = 20.036\ x^{0.0999}$, $R^2 = 0.30$, $p < 0.05$. Oxygen model equation: $y = 1.2119e^{-0.0496x}$, $R^2 = 0.29$, $p < 0.05$.

4.3.2 Soil temperature

The control plot had the lowest mean temperature of 19°C which was the same as the ambient atmospheric temperature followed by the landfill plot with topsoil, which had a significantly ($p<0.01$) higher temperature of 20.7°C. The landfill plot without topsoil had the significantly ($p<0.01$) highest mean soil temperature of 22.9°C.

The relationship between temperature and landfill gas in the soil of the control and experimental plots was assessed using a regression analysis. No significant variation in the gas measurements taken at different times during the experimental period was found, therefore a mean carbon dioxide, methane and oxygen value for all the measurements taken for each of the gas samplers was calculated. The mean gas measurement for each gas

sampler on all of the plots was compared with a single soil temperature reading made at each gas sampler. The ambient atmospheric air temperature at the time of the temperature measurements was 19°C. The relationship between the gases measured and the soil temperature was analysed using a exponential, linear, logarithmic and reciprocal regression in order to determine which equation fitted the data best in terms of the respective R^2 values.

A reciprocal regression (1/y= a+bx) was found to best describe the relationship that methane and carbon dioxide had with soil temperature. Methane had a R^2=0.70 and carbon dioxide a R^2=0.75. The temperature of the soil increased with the increasing concentrations of carbon dioxide (Figure 4.8) and methane (Figure 4.9) present. In the case of the relationship between oxygen and temperature in the soil (Figure 4.10), the temperature decreased exponentially with increasing oxygen concentrations (R^2=0.67). It can be concluded from the temperature results that methane and carbon dioxide were warm gases and were responsible for raising the soil temperatures above that of the ambient air temperature. The fact that oxygen has an opposite relationship with soil temperature was probably because oxygen concentrations were found to decrease with increasing methane and carbon dioxide concentrations (Figure 4.3 and 4.4). Therefore, the higher the oxygen levels, the lower the carbon dioxide and methane levels, and thus the lower the soil temperatures and the nearer the soil temperature would be to the ambient air temperature.

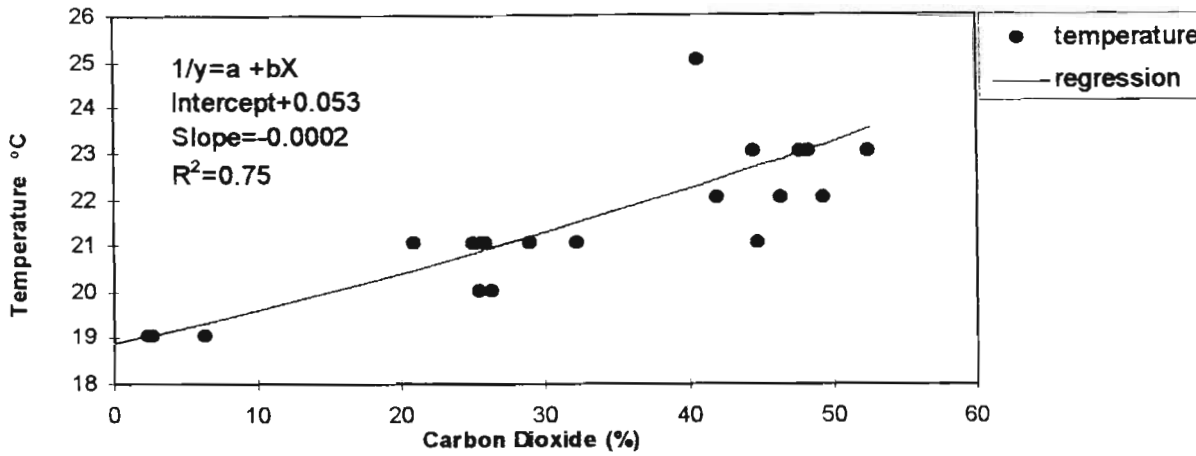


Figure 4.8: Relationship between % carbon dioxide and root zone temperature of the experimental plots

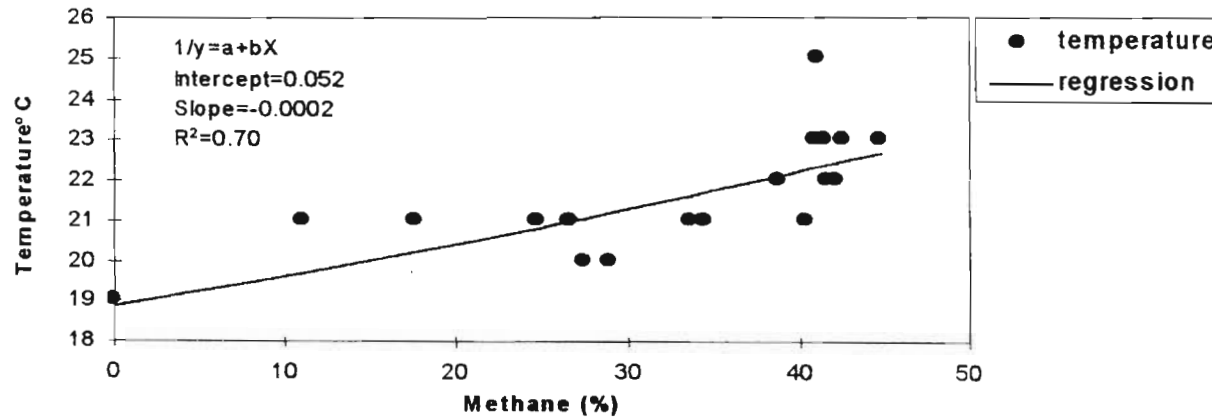


Figure 4.9: Relationship between % methane and root zone temperature of the experimental plots.

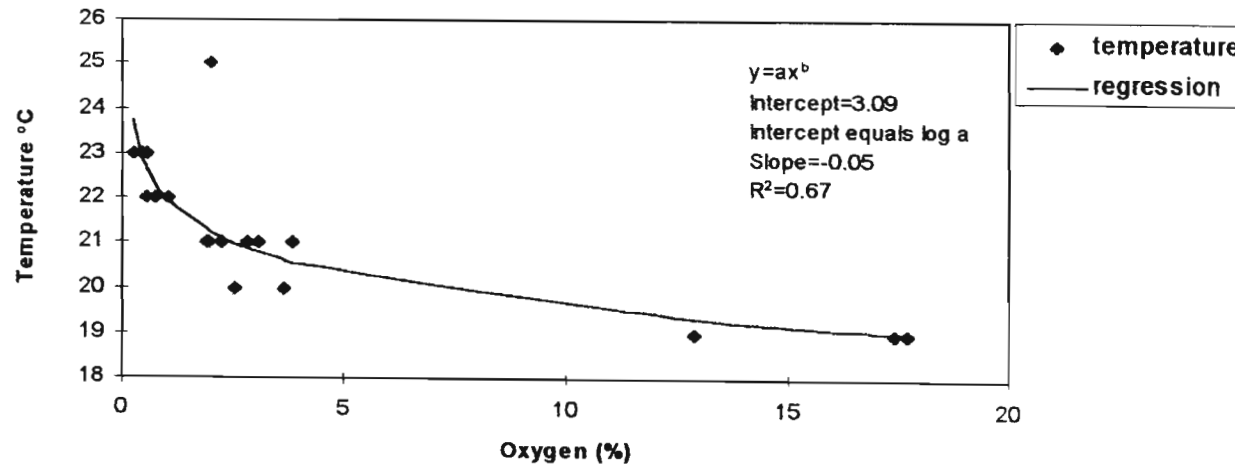


Figure 4.10: Relationship between % oxygen and root zone temperature of the experimental plots.

4.3.3 Soil chemical and physical characteristics

The measurement of the extractable (ammonium bicarbonate, pH 8) nutrients P, K, and Ca indicated that the landfill cover material (No topsoil plot) was not deficient in these nutrients by comparison to the topsoil used on the control and the experimental plot (Table 4.3). No significant differences ($p>0.05$) were found between the plots for P and Ca. However, K concentrations were significantly ($p<0.01$) (six fold) higher in the landfill cover material by comparison to the topsoil on the landfill topsoil plot and the control plot (Table 4.3). Mg concentrations were significantly ($p<0.05$) lower in the landfill cover material by comparison to the two plots with topsoil. There was no significant difference ($p>0.05$) in percentage clay and percentage organic carbon between the control and the experimental plots. There was no significant ($p>0.05$) difference in extractable acidity in any of the plots, however, the pH and conductivity of the plot on the landfill without topsoil was significantly ($p<0.01$) higher than the landfill plot with topsoil and the control plot.

No significant differences in the 13 soil variables in Table 4.3 were found between the topsoil on the control plot, and the topsoil on the landfill, except for soil moisture and manganese concentrations. The control plot, landfill plot with topsoil and the landfill plot without topsoil all had significantly ($p<0.01$) different soil moisture levels (Table 4.3). The control plot had the highest soil moisture by comparison to the plots on the landfill. The topsoil on the landfill had 1.5% less moisture than the control plot and a further 1.7% less moisture on the plot without topsoil was found.

Table 4.3: Physical and chemical properties of soil samples collected from the three experimental plots[1].

Parameter	Control plot	Topsoil Plot	No topsoil plot
Extractable Mn (mg/kg)	4.76 ±0.32[1] a[2]	31.02 ±3.1 b	22.49 ±2.6 b
Extractable Zn (mg/kg)	5.8 ±0.16 a	10.60 ±1.66 a	16.78 ±4.3 a
% moisture (by weight)	11.80 ±0.43 a	10.32 ±0.26 b	8.70 ±0.23 c
% Stone (by weight)	17.81 ±1.6 a	18.77 ±2.3 a	51.59 ±1.6 b
Extractable P (mg/kg)	16.4 ±0.7 a	18.1 ±0.7 a	12.7 ±2.7 a
Extractable K (mg/kg)	32.4 ±0.6 a	36.6 ±2.7 a	168.7 ±25.1 b
Extractable Ca (mg/kg)	1119.6 ±38.2 a	1003.8 ±76.8 a	990.67 ±86.5 a
Extractable Mg (mg/kg)	246.0 ±18.9 a	237.7 ±13.1 a	168.3 ±15.0 b
Organic carbon %	1.93 ±0.07 a	2.37 ±0.12 a	2.17 ±0.49 a
Clay %	24 ±0.58 a	23.33 ±1.20 a	26.33 ±3.28 a
Extrac. Acidity (Cmol/kg)	0.086 ±0.006 a	0.085 ±0.007 a	0.097 ±0.009 a
pH	7.43 ±0.11 a	7.24 ±0.05 a	8.14 ±0.12 b
Conductivity (mS/cm)	0.85 ±0.08 a	1.12 ±0.64 a	3.74 ±0.098 b

[1]Standard error of the mean (n=4)

[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Sheffe multiple range test ($p<0.01$), except for magnesium which was $p<0.05$.

An interesting difference in the chemistry of the topsoil on the landfill was a six fold higher ($p<0.01$) extractable (ammonium bicarbonate, pH 8) Mn concentration by comparison to the control plot (Table 4.3). One explanation for this is that there was an increase in Mn concentration in the topsoil after placement on the landfill. Interestingly, there was no significant difference ($p<0.01$) in Mn between the plots with and without topsoil on the landfill. Zinc concentrations were also found to have almost doubled in the topsoil placed on the landfill by comparison to the control, however the differences were not statistically significant ($p= 0.07$). Considering that the topsoil used on the control plot and that used on the landfill plot came from the same stock pile it can be concluded that the significant increase in Mn and possibly the increased amount of Zn was probably not due

to sampling. This indicates that the changes in the topsoil on the landfill may be due to an interaction between the topsoil and the underlying waste material below the plot.

A possible source of the high Mn levels may be leachate contamination of the soil. However, in this investigation drainage lines were installed to prevent leachate causing surface contamination of the experimental plots and there was no visual evidence of leachate contamination. The upward migration, by capillary action, of moisture, carrying Mn in solution, was also unlikely due to the high compaction and poor soil structure of the underlying waste and cover material. It may be possible that the upward migration of warm landfill gas carried a Mn condensate which was deposited in the topsoil layer as the gas cooled towards the soil surface. To investigate this further soil samples on the control and landfill plots were analysed for total metal concentrations using x-ray fluorescence. The results in Table 4.4 show no significant difference in total Mn concentrations between the plots. It is also interesting to note that in terms of the other metals measured there were also no significant differences between the topsoil on the control and the topsoil on the landfill. The only significant differences were between the landfill cover material and the topsoil which was used on both the control and landfill plot. The landfill cover material was found to have significantly ($p<0.05$) lower levels of metals (Al, Na, K, P, Nb, Y, Rb, Zr,Sr, Cr, Ba, Ga) in comparison to the topsoil, thus disproving the idea that the soils on the landfill were being influenced by metal-contaminated leachate or gas condensate.

Table 4.4: Total metal concentrations measured in the soil on the control and landfill plot with and without topsoil using x-ray fluorescence spectrometry (mg Kg^{-1}).

Element	Control plot		Topsoil plot		No topsoil	
Si	335286.4	±5913.9[1]	342522	±1157.6	340157.5	±8215.1
Al	56900.2	±1605.0 a[2]	55567.1	±281.0 ab	47137.9	±3681.3 b
Fe	32824.6	±4357.2	26214.5	±579.6	25825.8	±1213.8
Mn	633.0	±42.5	581.2	±42.0	517.0	±29.0
Mg	3911.1	±109.0	3617.9	±141.8	3816.7	±313.6
Ca	8721.4	±214.8	9240.6	±413.7	12855.5	±2581.9
Na	11299.0	±173.2 a	11819.5	±237.4 a	6880.8	±1492.5 b
K	20241.2	±596.4 a	21138.3	±610.0 a	14746.8	±1726.8 b
Ti	4365.2	±85.1	4341.6	±69.1	4224.8	±118.6
P	464.0	±11.0 a	435.4	±19.8 ab	380.5	±8.9 b
Nb	14.0	±0.4 a	13.6	±0.4 ab	11.6	±0.7 b
Y	37.7	±0.7 a	37.1	±0.7 a	28.9	±2.8 b
Rb	91.8	±1.1 a	92.4	±2.1 a	69.5	±7.3 b
Zr	568.0	±11.8 a	565.4	±22.6 a	385.2	±71.1 b
Sr	145.2	±1.7 a	145.3	±0.6 a	105.6	±10.1 b
U	3.1	±0.5	2.9	±0.3	2.7	±0.4
Th	10.8	±0.7	11.9	±0.6	10.9	±0.8
Zn	82.9	±1.0	79.4	±3.4	99.3	±7.6
Cu	13.7	±1.4	11.7	±2.2	19.4	±3.1
Ni	18.2	±0.3	17.1	±0.4	19.5	±1.3
Cr	80.6	±4.8 ab	71.1	±2.2 a	95.1	±6.6 b
V	88.1	±6.1	78.0	±1.9	94.0	±5.8
La	40.2	±1.0	41.7	±2.5	38.9	±8.8
Ba	743.9	±17.1 ab	776.2	±18.5 a	638.0	±48.9 b
Sc	19.0	±1.3	17.7	±0.8	19.6	±1.2
S	560.5	±85.2	1717.8	±536.9	2104.3	±806.0
Cd	3.7	±2.2	2.4	±1.9	1.7	±1.7
Pb	46.5	±2.0	39.8	±5.4	44.9	±3.2
Ga	13.9	±0.4 a	13.7	±0.2 a	11.5	±0.8 b
Co	16.0	±1.1	14.9	±1.5	13.1	±1.3
Ce	96.8	±3.8	111.1	±7.1	86.7	±13.2
Nd	40.7	±2.7	43.5	±2.5	39.6	±4.8
As	16.1	±1.4	14.8	±1.7	21.5	±2.9

[1]Standard error of the mean (n=4)

[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Sheffe multiple range test ($p<0.05$)

4.3.4 Relative performance of tree species

The trees on the plots that were removed from the data set because of a verified cause, such as those killed by insect infestation, stolen, or broken by the wind are shown in Table 4.5.

Table 4.5: The number of trees that were removed from the data set because of a verified cause.

Cause of death	Species	Plot	No. of trees
Insect damage	*Rhus lancea*	Topsoil	2
	Combretum erythrophyllum	Topsoil	1
	Syzygium cordatum	Topsoil	1
	Erythrina lysistemon	No topsoil	1
Wind damage	*Syzygium cordatum*	Topsoil	1
	Rhus lancea	Control	1
	Acacia xanthophloea	No topsoil	1
Stolen	*Barringtonia racemosa*	Topsoil	4

Table 4.5 indicates that insect damage was the main cause of verifiable tree death. The predominance of insect damage on the topsoil plot was most likely the result of a random single plant infestation that spread, and is unlikely to be related to differences in environmental conditions between the plots. Wind damage on the landfill plots could be expected, as on the landfill there was very little vegetation or topographical features to break the flow of air. The stealing of four *Barringtonia racemosa* from the fenced topsoil plot can only be used to illustrate the diversity of problems that can be encounted when trying to revegetate a landfill environment. Survival and health category measurements were collected for all seven *Barringtonia racemosa*, however, the trees were stolen before growth measurements were completed and, therefore, growth data for only three trees of this species from the landfill plot with topsoil were available. Theft is a general problem for landfill revegetation in South Africa. Most landfills support a population of people who

make a living from salvaging goods from the site, thus the temptation of newly planted trees which can be sold or used for medicinal purposes can be irresistible.

Tree mortality and health

Twelve trees were removed from the experimental data set for the assessment of the trees relative performance (Table 4.5). No deaths on the control plot were recorded within the first 7 months, however over the following 8 months *Acacia xanthophloea*, *Erythrina lysistemon*, *Rhus lancea* and *Acacia sieberiana* had a number of mortalities (Table 4.6). The effects of the stress of transplanting may have required a full growing season to become evident, possibly explaining the increase in mortality in the final 8 months of the experiment. *Acacia sieberiana* showed a similar increase in mortality with time on the control and experimental plots reinforcing the suggestion that transplanting stress may have contributed towards mortality. However, *Acacia xanthophloea* had a mortality that was higher on the control plot by comparison to that on the landfill experimental plots. It was also noted that the increase in mortality on the control plot, over the final eight months of the experiment for *Acacia xanthophloea*, *Erythrina lysistemon*, and *Rhus lancea* did not occur on the landfill experimental plots. This could suggest, especially for *Acacia xanthophloea*, that something other than transplanting stress might have been affecting these species on the control plot. This will be discussed in greater detail in the section on tree growth.

Table 4.6: Percentage of dead trees of each species on the control plot throughout the experimental period.

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0	0	0	0	14.3
Acacia xanthophloea	0	0	0	0	57
Barringtonia racemosa	0	0	0	0	0
Combretum erythrophyllum	0	0	0	0	0
Erythrina lysistemon	0	0	0	0	28.6
Harpephyllum caffrum	0	0	0	0	0
Hibiscus tiliaceus	0	0	0	0	0
Rhus lancea	0	0	0	0	14.3
Strelitzia nicolai	0	0	0	0	0
Syzygium cordatum	0	0	0	0	0
Mean	0	0	0	0	11.4

In terms of overall tree species mortality after 14 months the control plot experienced the lowest mortality of 11%, the landfill topsoil plot had a mortality of 23% and the landfill plot without topsoil had a mortality of 36% (Tables 4.6, 4.7 and 4.8). The higher mortality on the topsoil plot by comparison to the control plot, which received the same topsoil, suggested that changes in the soil characteristics of the topsoil on the landfill, or some other environmental variables, had a negative effect on the tree survival. However, the application of topsoil did have a beneficial effect on tree mortality, reducing it by 50% after 7 months when compared to the trees planted directly into the landfill cover material (Tables 4.7 and 4.8). It is important to note that over the final eight months, although the topsoil layer still resulted in lower tree mortality, mortality increased by 9% on the topsoil plot (Table 4.7), whilst it only increased by 4% on the no topsoil plot (Table 4.8). This may suggest that the ameliorative properties of the topsoil were reduced with time.

Table 4.7: Percentage of dead trees of each species on the Topsoil plot throughout the experimental period.

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0.0	0.0	0.0	0.0	28.6
Acacia xanthophloea	0.0	0.0	0.0	0.0	0.0
Barringtonia racemosa	0.0	0.0	0.0	0.0	0.0
Combretum erythrophyllum	0.0	0.0	16.7	16.7	16.7
Erythrina lysistemon	0.0	0.0	0.0	14.3	14.3
Harpephyllum caffrum	0.0	0.0	0.0	57.1	71.4
Hibiscus tiliaceus	0.0	0.0	0.0	0.0	0.0
Rhus lancea	0.0	0.0	0.0	0.0	0.0
Strelitzia nicolai	0.0	0.0	14.3	28.6	57.1
Syzygium cordatum	0.0	0.0	0.0	33.3	50.0
Mean			3.1	15	23.8

In terms of individual species survival on the landfill experimental plots *Hibiscus tiliaceus* and *Barringtonia racemosa* had no deaths on any of the plots (Table 4.7 and 4.8). *Combretum erythrophyllum* had only 14–17% mortality on the landfill plots. These mortality results suggested that *Barringtonia racemosa, Combretum erythrophyllum* and *Hibiscus tiliaceus* were relatively tolerant to the landfill conditions and the application of topsoil did not reduce the mortality of these species. This was confirmed by the health category data, which showed little change between the plots for these species (Figure 4.11).

Table 4.8: Percentage of dead trees of each species on the No topsoil plot throughout the experimental period

Species	20/1/97	31/1/97	4/4/97	31/7/97	6/4/98
Acacia sieberiana	0.0	0.0	42.9	42.9	57.1
Acacia xanthophloea	0.0	0.0	14.3	28.6	28.6
Barringtonia racemosa	0.0	0.0	0.0	0.0	0.0
Combretum erythrophyllum	0.0	0.0	0.0	14.3	14.3
Erythrina lysistemon	0.0	0.0	0.0	33.3	33.3
Harpephyllum caffrum	0.0	0.0	14.3	71.4	57.1
Hibiscus tiliaceus	0.0	0.0	0.0	0.0	0.0
Rhus lancea	0.0	0.0	14.3	28.6	28.6
Strelitzia nicolai	0.0	0.0	0.0	14.3	57.1
Syzygium cordatum	0.0	0.0	0.0	85.7	85.7
Mean	0	0	0	31.9	36.2

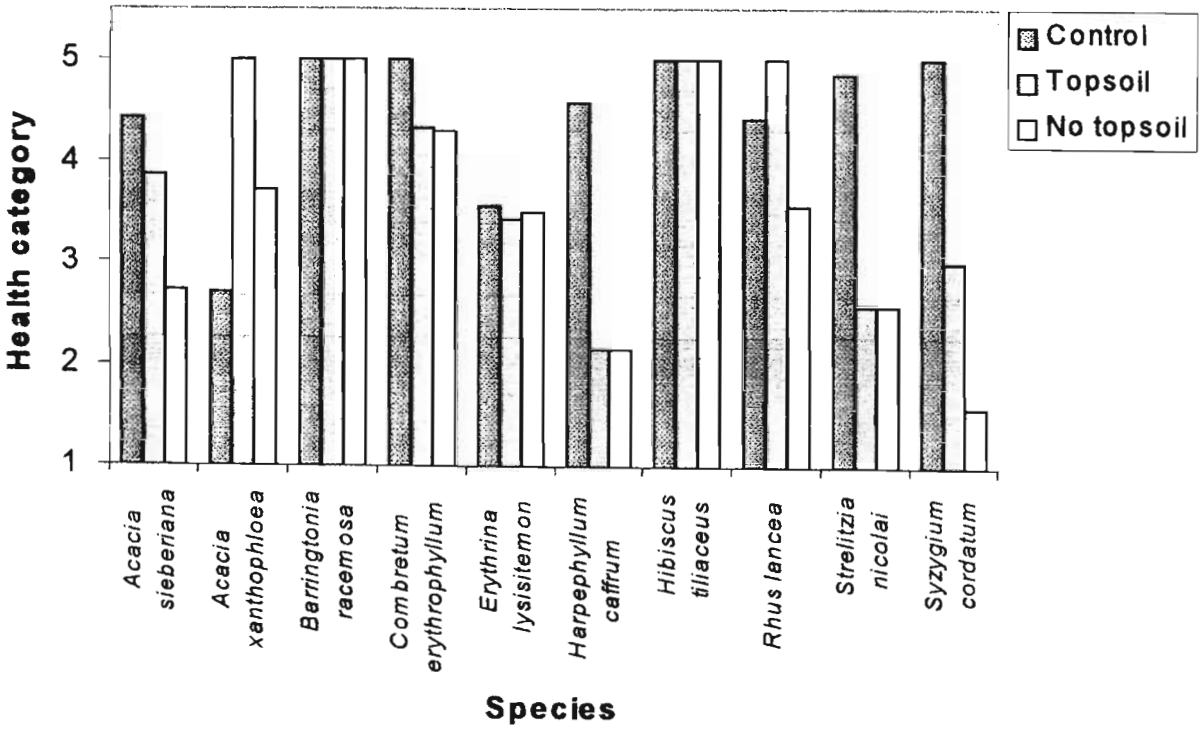


Figure 4.11: Mean health category based on the appearance of the individual plants recorded on the 6/4/98 at the end of the experiment.

Rhus lancea, Acacia xanthophloea, Erythrina lysistemon, Acacia sieberiana and *Syzygium cordatum* all appeared to benefit from the application of topsoil on the landfill and had fewer mortalities and better health on the topsoil plot in comparison with the no topsoil plot (Table 4.7 and 4.8; Figure 4.11). Of these species *Rhus lancea* and *Acacia xanthophloea* had a relatively low mortality on the no topsoil plot (28.6%) and with the application of topsoil no mortality was recorded for the 14 month experimental period. *Acacia sieberiana, Erythrina lysistemon* and *Syzygium cordatum* had a relatively high mortality on the no topsoil plot (57%), however, the application of a topsoil layer resulted in fewer mortalities 28%, 19% and 7% respectively. For some species, such as *Harpephyllum caffrum* and *Strelitzia nicolai,* which also had a high mortality on the no topsoil plot (57%), the application of topsoil did not result in fewer mortalities, in fact *Harpephyllyum caffrum* had a higher mortality on the topsoil plot (71%). It is also interesting to note that the percentage mortality of *Harpephyllum caffrum* declined by 14% between 31/7/97 and the 6/4/98, suggesting that one of the trees experienced re-growth and was not actually dead. In summary the results show that, in terms of tree mortality, there is a wide range of survival values on the landfill plots and the benefit of a topsoil layer over the landfill cover material is apparent for some species but not others.

Tree growth

The stem diameter and height growth of the individual trees was calculated by subtracting the measurements taken at the beginning of the experiment from that measured after 7 and 14 months of growth. For the whole data set the relationship between the increase in height over the experimental period and the original height when planted was investigated using linear regression. Data for all the individual trees on all the plots was used in the regression

and each species was analysed separately. The relative increase in stem diameter was assessed similarly. Surprisingly, no significant ($p>0.05$) relationship between the increase in stem dimensions and the original stem dimensions of the tree when planted was found for any of the species except *Acacia xanthophloea* and *Harpephyllum caffrum*. One may expect the increase in stem diameter and height to be greater for trees that were originally larger, because larger trees usually have greater productivity. This was true for the increase in stem diameter of *Harpephyllum caffrum*, which had a significant ($p<0.05$) positive relationship with the original stem diameter measured ($R^2=0.28$). However, the increase in stem height and diameter for *Acacia xanthophloea* had a significant ($p<0.05$) negative relationship with the original stem height and stem diameter measured, $R^2=0.23$ and $R^2=0.32$ respectively.

Even though only two species (*Harpephyllum caffrum* and *Acacia xanthophloea*) showed a relationship between the original size of the tree and size increase, the increase in stem diameter and height was expressed as a proportion of the original stem diameter and height (i.e. relative growth). It was also considered sensible to express aboveground biomass as a proportion of original stem height (i.e. relative biomass) in the following analysis.

The stem diameter and height growth as well as aboveground biomass and leaf area data was presented in two different ways for analysis. The data was firstly presented with all the dead plants included as zero values. For the stem diameter and height growth data some individual plants experienced negative growth which can be expected if plant health deteriorated and the plant tissue had lost water, these were also represented by zero values. Although the inclusion of the dead plants as zero growth could provide a good indication of individual species overall performance, it would mask information about the growth of

the surviving individuals. Therefore, the data was further presented for analysis with the dead plants removed from the data, and for the stem height and diameter data the negative growth values were also included, in order to assess the growth of the surviving trees.

With all the data for the different species combined there was no difference between the results using the two different methods of presenting the data, except for total leaf area, which will be discussed last. After the first 7 months there was a significantly ($p<0.01$) smaller height and diameter growth between the landfill plots and the control plot. The application of topsoil over the landfill cover material appeared to result in no significant ($p>0.05$) improvement on overall tree growth on the landfill (Figure 4.12). After 14 months the ameliorative effects of the topsoil started to become more apparent. There was no significant ($p>0.05$) difference in height growth between the control plot and the landfill plot which received topsoil, whilst the landfill plot which received no topsoil had a significantly ($p<0.01$) smaller growth in height (Figure 4.12a). In terms of stem diameter growth the ameliorative effects of the topsoil were also apparent after 14 months. There was a significantly better growth on the control plot then the landfill plot with topsoil however, the diameter growth of the trees planted without topsoil was significantly ($p<0.01$) less than the plots that received topsoil (Figure 4.12b).

The aboveground biomass data showed significantly reduced aboveground plant mass on the landfill when no topsoil layer was provided (Figure 4.12c). The total leaf area data also showed that plant growth was reduced by the landfill conditions, however, topsoil did not appear to reduce this effect, as seen in the stem growth and biomass results. When the dead plants were removed from the data set the total leaf area results were similar to the stem diameter and biomass results, showing reduced total leaf area on the no topsoil plot but no

significant ($p<0.01$) difference between the control and landfill topsoil plot. Therefore, the results generally suggest that tree growth was limited by the landfill conditions, however, the addition of a 1m-topsoil layer over the landfill cover material resulted in a marked improvement in growth.

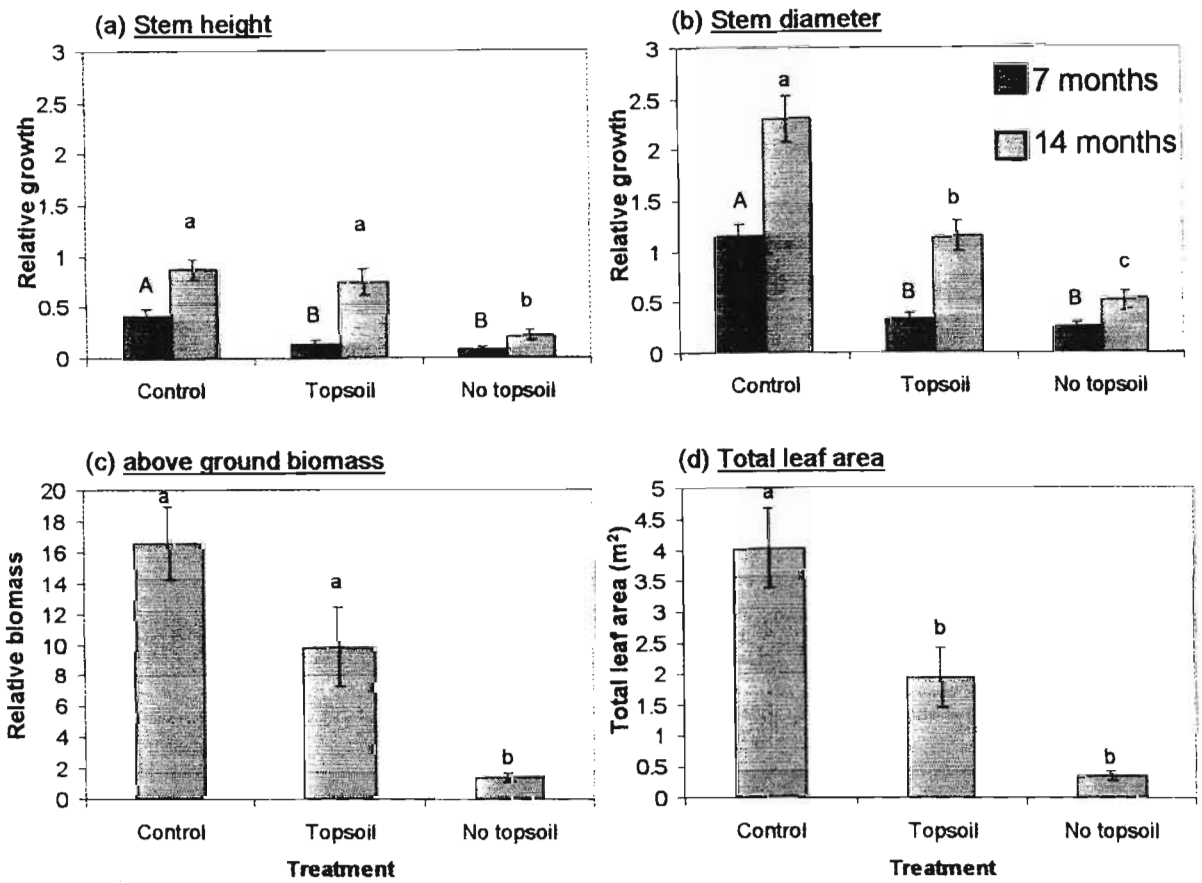


Figure 4.12: Growth response of trees to the landfill conditions. Mean relative height (graph a) and diameter (graph b) growth on control and experimental plots after 7 and 14 months. Significant differences ($p<0.01$) between plots within the 7 or 14 month category shown by a change in letter (upper case-7month; lower case-14month comparison). Mean above ground biomass after 14 months (graph c) and total leaf area after 14 months (graph d) significant differences ($p<0.01$) between treatments shown by change in letter. Error bars show standard error of the mean.

Similarly to the analysis of the combined species growth data the difference in individual species growth response data (i.e. stem diameter and height growth, biomass and total leaf area) between the two different methods of presenting the data was minimal, therefore unless specifically mentioned, the conclusions made from the two methods were the same. The key focus of the individual species stem growth results was on the data collected at 14 months, as it was considered more representative of the performance of the trees in the long term.

In the description of the results for the individual species, the species will be considered in two groups. The first group of 4 species is where there were few apparent effects of the landfill conditions (*Barringtonia racemosa; Acacia sieberiana; Erythrina lysistemon* and *Acacia xanthophloea*). The second group consists of the other six species (*Harpephyllum caffrum; Strelitzia nicolai; Syzygium cordatum; Combretum erythrophyllum; Hibiscus tiliaceus* and *Rhus lancea*). In the second group *Syzygium cordatum, Harpephyllum caffrum, and Strelitzia nicolai* showed no marked improvement in growth with the addition of topsoil on the landfill. However, *Combretum erythrophyllum, Hibiscus tiliaceus* and *Rhus lancea* all showed a marked improvement in growth when planted with the additional topsoil layer.

After 14 months the stem height growth, diameter growth, and biomass for *Barringtonia racemosa, Acacia sieberiana, Erythrina lysistemon,* and *Acacia xanthophloea* did not differ significantly ($p<0.05$) between the control and the landfill plots (Tables 4.9, 4.10, 4.11). However, the inherent variability of these measurements for *Acacia sieberiana* and *Erythrina lysistemon,* as indicated by the standard error of the mean height, diameter, biomass and leaf area data on the control were some of the highest of the 10 species

(Figure 4.13). The standard error expressed as a percentage of the mean variable measured in the control for both species, across all variables measured, was in excess of 36% (mean 50%), whilst most other species were well below 30% (Figure 4.13). Therefore the lack of significant difference in stem growth between the control plot and the landfill plots was probably due to high data variability and not necessarily indicative of a minimal growth effect of the landfill conditions. A higher number of replicates for *Erythrina lysistemon* and *Acacia sieberiana* may have resulted in better quality data for these species.

Table 4.9: Comparison of relative height increase between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative values=0 and dead plants=0.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	1.68 ±0.31[1] (7)[2] a[3]	1.76 ±0.68 (4) a	0.596 ±0.22 (7) a
Combretum erythrophyllum	0.56 ±0.06 (7) a	0.30 ±0.14 (6) ab	0.06 ±0.05 (7) b
Erythrina lysistemon	0.34 ±0.17 (7) a	0.027 ±0.02 (7) a	0.00 ±0.00 (6) a
Harpephyllum caffrum	1.29 ±0.23 (7) a	0.34 ±0.25 (7) b	0.00 ±0.00 (7) b
Hibiscus tiliaceus	1.47 ±0.16 (7) a	1.46 ±0.40 (7) a	0.37 ±0.12 (7) b
Rhus lancea	0.40 ±0.06 (6) a	0.23 ±0.07 (5) a	0.02 ±0.02 (7) b
Acacia sieberiana	0.80 ±0.37 (7) a	1.30 ±0.48 (7) a	0.50 ±0.24 (7) a
Strelitzia nicolai	0.70 ±0.14 (7) a	0.14 ±0.09 (7) b	0.04 ±0.04 (7) b
Syzygium cordatum	0.02 ±0.01 (7) a	0.00 ±0.00 (5) a	0.01 ±0.01 (7) a
Acacia xanthophloea	1.28 ±0.62 (7) a	1.86 ±0.32 (7) a	0.60 ±0.34 (6) a

[1]Standard error of the mean
[2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.10: Comparison of relative stem diameter increase between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative values=0 and dead plants=0.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	2.72 ±0.43[1] (7)[2] a[3]	2.73 ±0.90 (4) a	1.71 ±0.33 (7) a
Combretum erythrophyllum	1.91 ±0.14 (7) a	1.17 ±0.50 (6) ab	0.26 ±0.22 (7) b
Erythrina lysistemon	1.31 ±0.48 (7) a	0.44 ±0.17 (7) a	0.13 ±0.08 (6) a
Harpephyllum caffrum	0.94 ±0.16 (7) a	0.26 ±0.17 (7) b	0.01 ±0.01 (7) b
Hibiscus tiliaceus	3.39 ±0.14 (7) a	2.16 ±0.49 (7) b	0.69 ±0.18 (7) c
Rhus lancea	3.41 ±0.38 (6) a	1.62 ±0.40 (5) b	0.36 ±0.19 (7) c
Acacia sieberiana	1.02 ±0.51 (6) a	0.38 ±0.16 (7) a	0.38 ±0.31 (7) a
Strelitzia nicolai	2.87 ±0.28 (7) a	0.38 ±0.25 (7) b	0.39 ±0.24 (7) b
Syzygium cordatum	1.44 ±0.09 (7) a	0.33 ±0.21 (5) b	0.03 ±0.03 (7) b
Acacia xanthophloea	2.53 ±0.35 (7) a	3.88 ±1.84 (7) a	1.21 ±0.60 (6) a

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.11: Comparison of relative above ground biomass between the control and experimental plots i.e (biomass/ original height) after 14 months. Data presented for analysis with dead plants included as zero mass.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	4.20 ±1.3[1] (6)[2] a[3]	5.41 ±2.9 (4) a	1.01 ±0.4 (7) a
Combretum erythrophyllum	9.57 ±1.8 (7) a	5.98 ±3.1 (6) ab	1.59 ±0.8 (6) b
Erythrina lysistemon	6.10 ±3.1 (7) a	1.17 ±0.6 (7) a	0.39 ±0.2 (6) a
Harpephyllum caffrum	10.59 ±2.5 (7) a	2.17 ±1.6 (7) b	0.16 ±0.1 (7) b
Hibiscus tiliaceus	54.96 ±7.0 (7) a	47.70 ±17.0 (7) a	3.63 ±1.4 (7) b
Rhus lancea	29.82 ±4.7 (5) a	8.08 ±1.8 (5) b	1.45 ±0.8 (7) b
Acacia sieberiana	16.13 ±8.8 (5) a	9.28 ±3.3 (7) a	2.53 ±1.6 (7) a
Strelitzia nicolai	6.49 ±1.4 (7) a	1.07 ±0.7 (7) b	0.57 ±0.3 (7) b
Syzygium cordatum	10.94 ±1.6 (7) a	1.65 ±1.0 (5) b	0.27 ±0.3 (7) b
Acacia xanthophloea	18.73 ±9.4 (7) a	10.60 ±2.2 (7) a	2.18 ±1.2 (6) a

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.12: Mean total leaf area of the surviving trees of different species between the experimental plots (Dead plants removed). (Area in m^2)

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	0.60 ±0.17[1] (6)[2] a[3]	0.73 ±0.36 (4) a	0.17 ±0.08 (7) a
Combretum erythrophyllum	2.89 ±0.71 (7) a	2.65 ±1.15 (5) a	0.67 ±0.37 (6) a
Erythrina lysistemon	2.52 ±1.49 (5) a	0.42 ±0.27 (6) a	0.066 ±0.01 (4) a
Harpephyllum caffrum	2.00 ±0.46 (6) a	1.33 ±0.82 (2) a	0.04 ±0.01 (3) a
Hibiscus tiliaceus	13.82 ±1.9 (7) a	10.63 ±3.2 (7) a	1.09 ±0.53 (7) b
Rhus lancea	13.37 ±1.9 (6) a	4.07 ±0.90 (5) b	1.02 ±0.55 (5) b
Acacia sieberiana	1.18 ±0.67 (5) a	1.23 ±0.28 (5) a	0.72 ±0.38 (3) a
Strelitzia nicolai	1.81 ±0.34 (7) a	0.92 ±0.51 (3) a	0.48 ±0.16 (3) a
Syzygium cordatum	3.91 ±0.68 (7) a	1.09 ±0.52 (3) a	0.39 ±0.0 (1) a
Acacia xanthophloea	3.32 ±0.25 (3) a	0.98 ±0.23 (7) b	0.28 ±0.15 (4) b

[1]Standard error of the mean. [2]Sample size (n)

[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

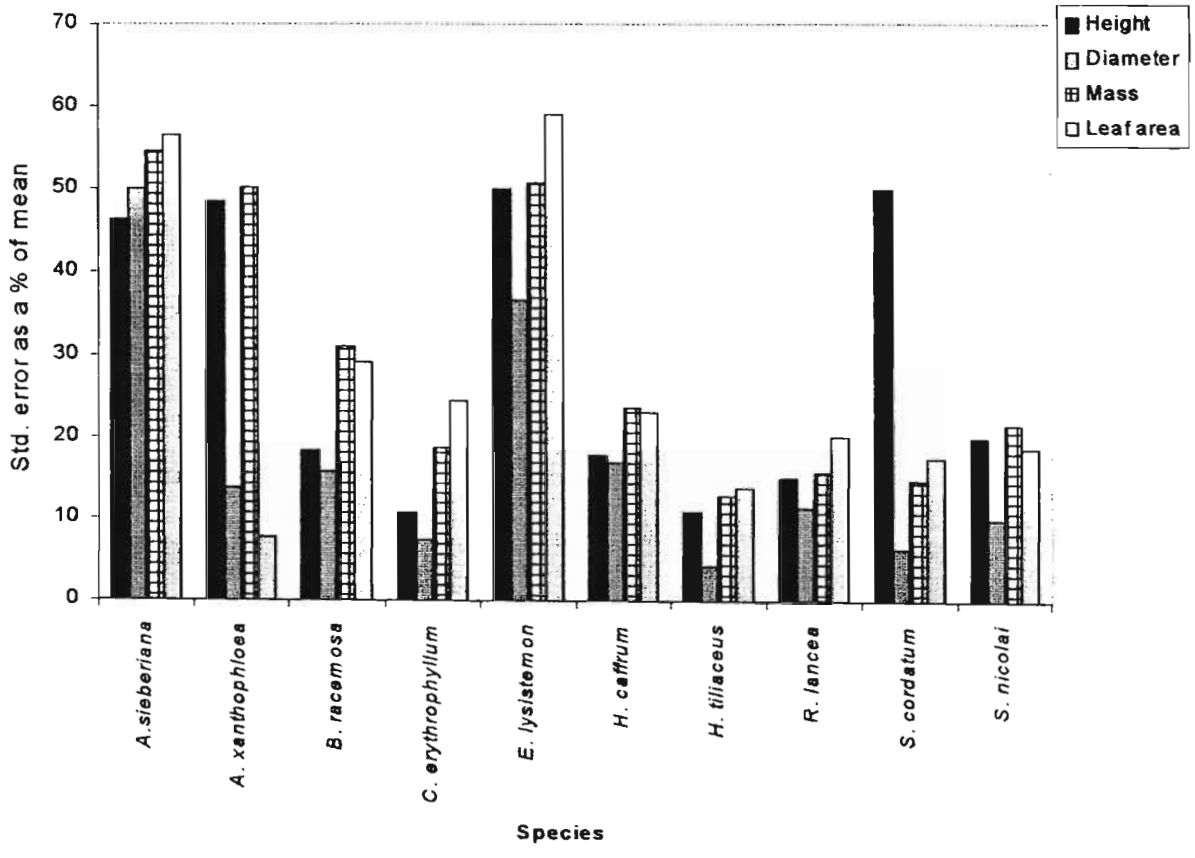


Figure 4.13: The inherent variability of each growth variable measured for each species (height and diameter growth, aboveground biomass and total leaf area), shown by expressing the standard error of the control mean of each growth variable as a percentage of that mean for each species.

Although *Acacia xanthophloea* also showed no significant difference in stem diameter growth, height growth and biomass there were also some problems with the data. There were unexplained mortalities on the control plot (Table 4.6), which when shown as zero growth in the data set resulted in low growth values (Tables 4.9, 4.10, 4.11). Thus, although growth of the species was reduced by the landfill conditions no significant difference in height growth, diameter growth as well as biomass between the control and landfill plots was found. However, by specifically focusing the comparison of growth between plots on the surviving plants only the analysis revealed that the height and diameter of *Acacia xanthophloea* as well as *Erythrina lysistemon* were reduced by the landfill conditions without topsoil (Tables 4.13 and 4.14).

The topsoil layer on the landfill slightly improved the height growth for *Erythrina lysistemon* and *Acacia xanthophloea.* It also improved the diameter growth of *Erythrina* but showed no significant improvement in diameter growth of *Acacia xanthophloea* (Tables 4.13 and 4.14). No further significant differences in *Erythrina* growth were found, however, the above ground biomass of the surviving *Acacia xanthophloea* trees was reduced by the landfill conditions and the topsoil layer resulted in no significant mass increase (Table 4.15). Furthermore, there was also a significant reduction in *Acacia xanthophloea* total leaf area on the landfill plots relative to the control and the topsoil layer appeared to provide little improvement (Table 4.12). Thus, of the species that showed no significant difference in growth in Table 4.9, 4.10, and 4.11, only the growth of *Barringtonia racemosa* was unaffected by the landfill conditions. The other species, *Acacia xanthophloea* and *Erythrina lysistemon* both showed evidence of reduced growth, whilst the variability of data for *Acacia sieberiana* was too high for reliable conclusions to be reached.

Table 4.13: Comparison of relative height increase of surviving plants between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative growth values included and dead plants removed.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	1.68 ±0.31[1] (7)[2] a[3]	1.76 ±0.68 (4) a	0.54 ±0.25 (7) a
Combretum erythrophyllum	0.56 ±0.06 (7) a	0.23 ±0.23 (5) ab	-0.24 ±0.18 (6) b
Erythrina lysistemon	0.47 ±0.22 (5) a	-0.14 ±0.10 (5) ab	-0.39 ±0.14 (4) b
Harpephyllum caffrum	1.29 ±0.23 (7) a	1.18 ±0.56 (2) a	-0.10 ±0.09 (2) a
Hibiscus tiliaceus	1.47 ±0.16 (7) a	1.46 ±0.40 (7) a	0.37 ±0.12 (7) b
Rhus lancea	0.40 ±0.06 (6) a	0.23 ±0.07 (5) a	-0.21 ±0.14 (5) b
Acacia sieberiana	0.83 ±0.49 (5) a	1.82 ±0.49 (5) a	1.17 ±0.13 (3) a
Strelitzia nicolai	0.70 ±0.14 (7) a	0.19 ±0.31 (3) a	-0.08 ±0.21 (3) a
Syzygium cordatum	-0.01 ±0.03 (7) a	-0.11±0.06 (2) a	0.06 ±0.00 (1) a
Acacia xanthophloea	2.99 ±0.43 (3) a	1.86 ±0.32 (7) ab	0.90 ±0.45 (4) b

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.14: Comparison of relative diameter increase of surviving plants between the control and experimental plots i.e (final height - original height)/ original height) after 14 months. Data presented for analysis with negative growth values included and dead plants removed.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	2.72 ±0.43[1] (7)[2] a[3]	2.73 ±0.90 (4) a	1.71 ±0.32 (7) a
Combretum erythrophyllum	1.91 ±0.14 (7) a	1.41 ±0.54 (5) ab	0.23 ±0.27 (6) b
Erythrina lysistemon	1.83 ±0.49 (5) a	0.60 ±0.19 (5) ab	0.19 ±0.11 (4) b
Harpephyllum caffrum	0.94 ±0.16 (7) a	0.91 ±0.07 (2) ab	-0.01 ±0.09 (2) b
Hibiscus tiliaceus	3.40 ±0.14 (7) a	2.16 ±0.49 (7) b	0.69 ±0.18 (7) c
Rhus lancea	3.41 ±0.38 (6) a	1.62 ±0.40 (5) b	0.50 ±0.25 (5) b
Acacia sieberiana	1.08 ±0.66 (5) a	0.53 ±0.18 (5) a	0.88 ±0.68 (3) a
Strelitzia nicolai	1.86 ±0.69 (7) a	0.83 ±0.52 (3) a	0.90 ±0.41 (3) a
Syzygium cordatum	1.44 ±0.09 (7) a	0.83 ±0.15 (2) b	0.17 ±0.00 (1) b
Acacia xanthophloea	9.07 ±0.48 (3) a	2.53 ±0.35 (7) b	1.81 ±0.74 (4) b

[1]Standard error of the mean.
[2]Sample size (n)
[3]a,b,c: The mean values in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

Table 4.15: Comparison of relative above ground biomass of surviving plants between the control and experimental plots i.e (biomass/ original height) after 14 months. Data presented for analysis with dead plants excluded from the data.

Species	Control plot	Topsoil plot	No topsoil plot
Barringtonia racemosa	4.20 ±1.3[1] (6)[2] a[3]	5.41 ±2.9 (4) a	1.01 ±0.4 (7) a
Combretum erythrophyllum	9.57 ±1.8 (7) a	7.17 ±3.5 (5) a	1.91 ±0.9 (5) a
Erythrina lysistemon	8.54 ±3.9 (5) a	1.37 ±0.6 (6) a	0.58 ±0.2 (4) a
Harpephyllum caffrum	10.59 ±2.5 (7) a	7.45 ±4.1 (2) a	0.37 ±0.1 (3) a
Hibiscus tiliaceus	54.96 ±7.0 (7) a	47.70 ±17.0 (7) a	3.63 ±1.4 (7) b
Rhus lancea	29.82 ±4.7 (5) a	8.08 ±1.9 (5) b	2.03 ±1.0 (5) b
Acacia sieberiana	20.16 ±10.2 (4) a	13.0 ±3.3 (5) a	5.90 ±2.8 (3) a
Strelitzia nicolai	6.49 ±1.4 (7) a	2.50 ±1.4 (3) a	1.32 ±0.6 (3) a
Syzygium cordatum	10.94 ±1.6 (7) a	4.13 ±0.7 (2) a	1.88 ±0.0 (1) a
Acacia xanthophloea	43.70 ±8.3 (3) a	10.60 ±2.2 (7) b	3.27 ±1.5 (4) b

[1]Standard error of the mean. [2]Sample size (n)
[3]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

The other six species (*Harpephyllum caffrum; Strelitzia nicolai; Syzygium cordatum; Combretum erythrophyllum; Hibiscus tiliaceus* and *Rhus lancea*) showed a more marked reduction in growth on the landfill. However, the species growth response to the additional topsoil layer on the landfill was variable.

Although the application of topsoil had a beneficial effect on growth for some species, the growth of *Harpephyllum caffrum*; *Strelitzia nicolai* and *Syzygium cordatum* was not improved. These species experienced high mortalities and the number of surviving plants on the landfill plots on which growth measurements could be made was less than or equal to 3. Therefore, primarily due to the low number of remaining replicates, analysis of the growth variables measured on surviving individuals generally resulted in no significant difference between plots ($p>0.05$). However, the analysis of the data that included dead

plants as zero clearly showed a significant reduction in species performance with or without topsoil on the landfill for *Harpephyllum caffrum*; *Strelitzia nicolai* and *Syzygium cordatum*. *Syzygium cordatum* did however have an unusually high inherent variability in height growth (50%) that resulted in no significant difference in height growth between the control and landfill plots (Figure 4.13). This was not considered indicative that growth was not affected by the landfill conditions, it was more likely that the high data variability in the data was responsible for the conclusion. However, it was clear that *Syzygium cordatum*, *Harpephyllum caffrum*, and *Strelitzia nicolai* performed poorly on the landfill and a topsoil layer was of little benefit.

Some species, such as *Hibiscus tiliaceus* and *Rhus lancea* showed a clear reduction in growth (i.e. stem diameter height and diameter, biomass, leaf area) when planted directly into the landfill cover material. However, the use of topsoil relative to no topsoil on the landfill resulted in significant ($p<0.05$) increase within all the growth variables measured for *Hibiscus tiliaceus* (Tables 4.9, 4.10, 4.11 and 4.12). *Rhus lancea* also showed a significant ($p<0.05$) increase in stem diameter and height growth on the topsoil plot relative to the no topsoil plot (Tables 4.9 and 4.10). However, unlike *Hibiscus* the topsoil layer provided no significant ($p>0.05$) improvement of tree biomass and total leaf area (Tables 4.11 and 4.12).

The topsoil layer also appeared to improve the growth of *Combretum erythrophyllum*, as there was no significant ($p>0.05$) difference in stem height growth, diameter growth, or mass between the topsoil plot and control plot. However, the improvement was minimal, as these variables on the topsoil plot were also not significant ($p>0.05$) different on the landfill plot without topsoil (Tables 4.9, 4.10 and 4.11). Interestingly, the *Combretum* leaf

area data did not differ between the control and the experimental plots (Table 4.12) and further analysis of the measured growth variables including only surviving plants showed no significant ($p>0.05$) difference in aboveground mass either (Table 4.15). It was apparent that species responded differently to the topsoil layer on the landfill, however, for *Hibiscus tiliaceus, Rhus lancea* and *Combretum erythrophyllum* the topsoil layer appeared to provide some improvement in growth.

In summary, there appeared to be a large variation in the growth response of different tree species to landfill conditions. The results indicated that *Barringtonia racemosa* growth was the least influenced by the landfill conditions. Although *Acacia sieberiana* also showed very little significant difference in growth between the plots, this was attributed to the relatively high data variability and not species tolerance to landfill conditions. The growth of *Hibiscus tiliaceus, Rhus lancea, Combretum erythrophyllum, Acacia xanthophloea* and *Erythrina lysistemon* was reduced by the landfill conditions, however, a topsoil layer over the landfill cover material helped to improve the trees growth. The growth of *Harpephyllum caffrum, Strelitzia nicolai* and *Syzygium cordatum* were significantly reduced by the landfill conditions with or without topsoil, indicating that these species were sensitive to the landfill conditions.

Although the data for some species was a difficult to interpret the following ranking of all 10 species, from least sensitive to most sensitive to the landfill environment was suggested: *Barringtonia racemosa, Combretum erythrophyllum, Acacia xanthophloea, Hibiscus tiliaceus; Rhus lancea; Erythrina lysistemon; Acacia sieberiana; Strelitzia nicolai; Syzygium cordatum* and *Harpephyllum caffrum.* The general ranking position of some species may vary slightly with the addition of topsoil, such as *Hibiscus* which should shift

up one position in the ranking because it responded relatively well to topsoil layer. Relative positions of *Erythrina lysistemon* and *Acacia sieberiana* could be questionable due to the unreliable nature of their data.

4.3.5 Species root morphology

The maximum rooting depth of the ten species after 14 months of growth, under normal conditions (i.e. the control plot) was 70cm. However, even with the use of the same topsoil type and depth the maximum rooting depth on the landfill was lower (40cm). Without topsoil on the landfill the maximum rooting depth was even shallower (20cm), this was probably due to landfill gas as well as poor soil structure (Figure 4.14). Table 4.16 shows the overall mean density of the roots per m^2 on the landfill topsoil (69.9 ±10.0 n=10) and no topsoil plots (30.6 ±4.4 n=10) were significantly ($p<0.05$) lower in comparison to the control plot (231.2 ±37.4 n=10). The lack of a significant ($p>0.05$) difference in overall root density between the landfill plots suggested that the topsoil layer did not significantly reduce the impact of the landfill environment on root density.

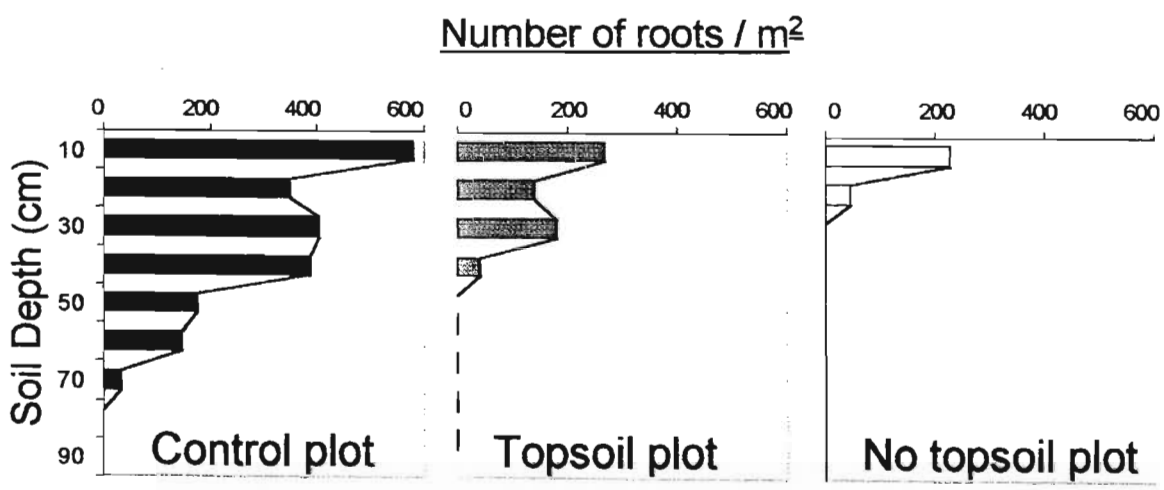


Figure 4.14:Mean root density (area) with soil depth for all species combined within each experimental plot (n=10)

Table 4.16: Total density of roots and the percentage of the total number of roots counted in the profile walls that were found in the top 10cm of the soil

	Control plot	Topsoil Plot	No topsoil plot
Mean Total density	231.2 ±37.4[1] a[2]	69.9 ±10.0 b	30.6 ±4.4 b
Mean % in top 10cm	32.7 ±6.7 a	48.4 ±6.8 a	86.1 ±4.7 b

[1]Standard error of the mean (n=10)
[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

However a significantly ($p<0.05$) lower percentage of the total number of roots recorded in each profile were found within the upper 10cm of the soil on the landfill topsoil plot relative to the landfill plot with no topsoil (Table 4.16). Considering there was no significant difference in root density between the topsoil and no topsoil landfill plots the difference in rooting depth in the no topsoil landfill plot was unlikely to be a function of reduced root growth but was an actual shallower-rooting plant response. This indicated that the topsoil layer on the landfill generally allowed for a greater proportion of roots to be deeper within the soil which could help to alleviate drought and nutrient stress usually associated with surface soil layers in a seasonal rainfall climate.

Although the data was very limited (n=1) individual species root response to the landfill with and without topsoil appeared to be variable and species specific (Table 4.17). There did not appear to be any relationship between the overall species performance and the degree to which root density was reduced by the landfill conditions. This relationship was tested by regression analysis of the percentage reduction in root density on the landfill plots relative to the control verse the above ground biomass as a percentage of the mean control biomass for each species. For the analysis *Erythrina lysistemon*, *Acacia xanthophloea*, and *Acacia sieberiana* were removed from the data set because the species

mass data was highly variable (Figure 4.13). The result of the linear regression showed no significant relationship between the reduction in root density and the mass of the species on the landfill topsoil plot ($p=0.84$; $R^2=0.009$) or no topsoil plot ($p=0.185$ $R^2=0.32$). The lack of a clear relationship between root density and differential species performance suggests that the ability to have a greater root density on the landfill was probably not the key reason for differential species performance.

Table 4.17: Total density of roots and the percentage of the total number of roots counted in the profile walls that were found in the top 10cm of the soil for each species on the three experimental plots.

Species	Control plot		Topsoil plot		No topsoil plot	
	Density	%	Density	%	Density	%
Acacia sieberiana	266.7	48.4	36.7	44.1	56.7	100.0
Acacia xanthophloea	302.2	10.9	80.0	73.5	23.3	91.3
Barringtonia racemosa	95.6	30.0	41.1	57.6	26.7	58.8
Combretum erythrophyllum	305.6	55.9	40.0	27.8	43.3	81.0
Erythrina lysistemon	365.6	60.6	37.8	32.0	25.6	100.0
Harpephyllum caffrum	423.3	24.6	80.0	16.4	27.8	92.9
Hibiscus tiliaceus	101.1	25.7	103.3	37.5	14.4	88.0
Rhus lancea	131.1	12.0	122.2	58.3	15.6	87.2
Strelitzia nicolai	115.6	55.8	55.6	86.5	23.3	62.5
Syzygium cordatum	197.8	2.8	101.1	50.5	46.7	100.0

[1]Standard error of the mean
[2]a,b,c: The means in the rows across the table followed by different letters are significantly different with a Scheffe Multiple Range test ($p<0.05$)

However it was noted in Table 4.17 that a large percentage of the total number of roots counted for each tree, on the no topsoil plot, were within the top 100mm of soil. The relationship between the percentage of the total number of roots in the top 10cm of soil and the above ground biomass as a % of the control mass of each species on the landfill topsoil and no topsoil plot was investigated using linear regression. As with the previous

regression analysis *Erythrina lysistemon, Acacia sieberiana* and *Acacia xanthophloea* were removed from the data set. A significant ($p=0.042$; $R^2=0.6$) negative linear relationship was found between species mass and the percentage roots in the first 10cm of soil on the landfill no topsoil plot (Figure 4.15). This suggested that the species which performed badly on the landfill no topsoil plot had a large percentage of their roots in the upper 10cm of soil whilst those which performed better were deeper rooting. This negative relationship was not clear on landfill topsoil plot and no significant linear relationship was found ($p=0.1$; $R^2=0.008$). This lack of a clear relationship would be expected as there was a significantly lower % of roots in the top 10cm of soil on the topsoil plot (Table 4.16) and the overall performance of the tree on the topsoil plot was better than the no topsoil plot.

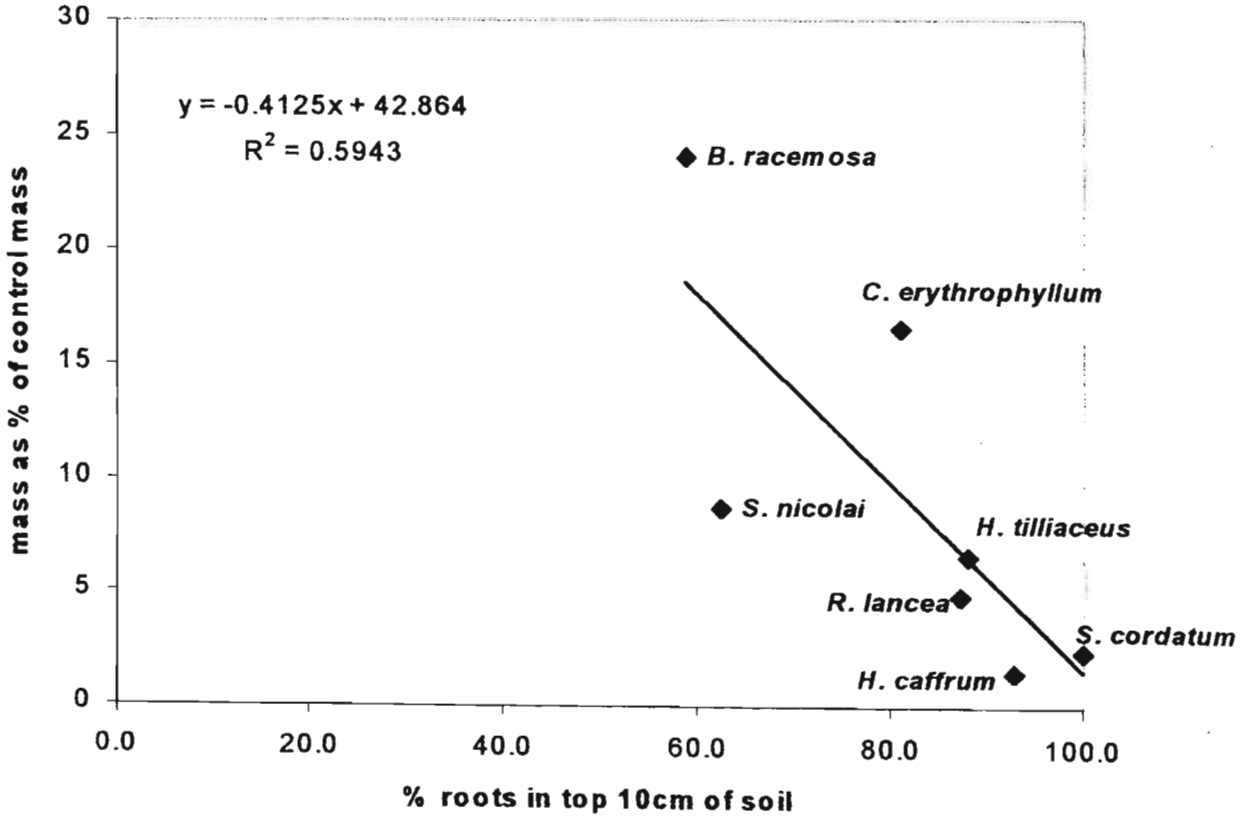


Figure 4.15: Linear regression showing the relationship between the percentage of the total number of roots in the top 10cm of soil and the above ground biomass expressed as a percentage of the control biomass for each species on the no topsoil plot.

The results suggested that for all the species the landfill conditions resulted in lower rooting densities and shallower rooting depths, however, the species which performed best on the landfill were probably able grow a greater proportion of their roots at a relatively greater depth.

4.4 DISCUSSION

The small loss of trees due to insect damage and wind can be expected in any field experiment or revegetation project. Loss of trees planted on landfills due to theft is not unique to the Bisasar Road landfill, Mackay & Richardson, (1996) reported a loss of 1.4% of 864 trees planted on Whabbs Tip, Merseyside, England. Although, the loss of trees due to theft was not large it is interesting to note that only one species, *Barringtonia racemosa*, was stolen. This species could have been targeted for resale as an ornamental plant for gardens or more likely it was taken for medicinal purposes. Extracts from *Barringtonia racemosa* plant tissues are reported to be used for making emetic solution against malaria as well as treatment of skin disease and stomach ache (Hutchings *et al*, 1996).

The overall much higher mortality of trees on the landfill by comparison to the control site confirmed the findings of many other researchers that the landfill environment presents a formidable challenge to vegetation growth, especially that of trees (Chan *et al* 1991; Lan & Wong 1994; Dobson & Moffat 1994; Ettala *et al* 1988; Flower *et al* 1981; Gill 1970; Gilman *et al* 1981; Insley & Carnell 1982; Leone *et al* 1983; Leone *et al* 1977; Moffat & Houston 1991). The reduction in the severity of the landfill environment by the application of a topsoil layer was clearly reflected in the lower mortality and improved stem growth of

some of the species. Insley and Carnell, (1982) also found that an additional layer of soil over the standard compacted cover material improved tree growth and survival.

In terms of the individual species the mortality and growth results showed that the sensitivity of plants to the landfill environment was species specific, confirming the findings of a number of other investigators from around the world (Leone *et al* 1983; Mackay & Richardson 1996; Chan *et al* 1991; Gilman *et al* 1981; Leone *et al* 1977; Flower *et al* 1981; Ettala 1988; Robinson *et al* 1992). For example, the landfill environment appeared to have no effect on the mortality and growth of *Barringtonia racemosa* irrespective of the use of topsoil. Whilst other species such as *Syzygium cordatum*, *Strelitzia nicolai* and *Harpephyllum caffrum* had very high mortalities and reduced growth with or without an additional topsoil layer. However, there were species with an intermediate response to the landfill environment such as *Combretum erythrophyllum*, *Hibiscus tiliaceus* and *Rhus lancea*. They had few or no mortalities and although growth was reduced in the trees planted directly into the landfill cover material, the use of a topsoil layer was found to ameliorate this effect. Unlike *Combretum* and *Hibiscus* there was evidence that the benefit of a topsoil layer for *Rhus lancea* was more limited.

The overall performance of the species on the landfill resulted in the following ranking from best species to worst: *Barringtonia racemosa, Combretum erythrophyllum, Acacia xanthophloea, Hibiscus tiliaceus; Rhus lancea; Erythrina lysistemon; Acacia sieberiana; Strelitzia nicolai; Syzygium cordatum* and *Harpephyllum caffrum.* The general ranking position of some species may vary slightly with the addition of topsoil, such as *Hibiscus* which should improve its ranking by one position because it responded relatively well to

topsoil layer. Relative positions of *Erythrina lysistemon* and *Acacia sieberiana* could be questionable due to the unreliable nature of their data.

It is interesting to note that almost all of the ten species used in this experiment have a normal habitat associated with rivers or water courses (Palgrave, 1984; Pooley, 1994). However, two of the species which performed best, namely *Barringtonia racemosa* and *Hibiscus tiliaceus* are usually found fringing swamps or tidal lagoons (Palgrave, 1984; Pooley, 1994). Therefore, the normal habitat of these trees would often be flooded, resulting in anaerobic soil conditions similar to those found on the landfill, thus, possibly explaining the better performance of these species in the experiment. A similar association was found by Arthur *et al* (1981) between flooding-tolerant species and tolerance to landfill gases. Therefore, the flood-tolerant characteristics of tree species are worthy of further investigation for the selection of species suitable for landfill revegetation.

The root morphology data also provided some insight into the differential species performance on the landfill. It was apparent that the landfill with or without a topsoil layer reduced the density of tree roots. Gilman *et al* (1981) also noted reduced tree root growth in landfill field investigations, which was correlated with high levels of soil CO_2 and low soil O_2. The effect of reduced root growth in landfill soils caused by elevated soil CO_2 and low soil O_2 was further confirmed by laboratory based experiments conducted by Marchiol *et al* (2000).

A shallower rooting response in landfill environments is not an uncommon phenomenon (Chan *et al* 1991; Gilman *et al* 1982; Gilman *et al* 1981). It has been suggested that those species which could direct their root growth towards the surface and away from the landfill

gas source are more successful in growth on landfills (Gilman *et al* 1982; Gilman *et al* 1981). It has also been noted that trees with a shallower root system are more susceptible to water stress in landfill soils, which usually have poor structure (Chan *et al* 1991). However, it has been suggested that species, which are normally shallow rooted, should be relatively tolerant of the low soil moisture conditions and their shallow-rooting behaviour would reduce the exposure to landfill gas found at higher concentrations deeper in the soil (Gilman, 1989).

In this experiment the opposite trend was apparent. Although all species showed a change in root growth towards the surface, it was apparent that those species that could maintain a greater proportion of their total number of roots deeper within the soil were more successful (Figure 4.15). This would suggest that the avoidance of the low soil moisture levels usually associated with the surface soil layers combined with the ability to continue deeper rooting into soils which have high concentrations of landfill gas may be a better strategy resulting in greater survival and growth of trees on landfills. The ability to maintain a greater rooting depth and avoid the soil moisture deficit in the surface soil layer was also identified as beneficial for species survival on landfills by Liang *et al* (1999). However, the work by Liang addressed soil compaction as the key factor that was limiting rooting depth and not landfill gas. Nonetheless shallower rooting which was previously considered beneficial for survival and growth on landfills may be detrimental to plant survival in climates with prolonged seasonal dry periods.

The results indicated that plant rooting depth was controlled by the soil gas composition confirming the work of Chan *et al* (1991), Gilman *et al* (1982) and Gilman *et al* (1981). By comparison of the topsoil plot and the no topsoil plot rooting depth appeared to be limited

at similar concentrations of landfill gas. The soil depth at which methane was 53-57%, CO_2 20-27% and O_2 1-2% was the common limit at which roots would no longer penetrate. These CO_2 and O_2 concentrations were comparable with those found by Gilman *et al* (1981) who noted that roots stopped growing or were redirected towards the surface when CO_2 was 8-23% and O_2 was 4-18%.

It has been noted since as early as 1946 that the effect of high carbon dioxide concentrations in the soil atmosphere varies greatly between plant species (Flower *et al* 1981; Geisler, 1963; Leonard & Pinkard, 1946; Stolwijk & Thimann, 1957). It is important that the relationship between soil gases in landfill soils is clearly understood. For example, although there is no evidence to show that methane is phytotoxic it does play a significant role by reducing the concentrations of oxygen by displacement and by the bacterial oxidation of methane into carbon dioxide. Thus, it may contribute to the increasing of concentrations of the more toxic carbon dioxide (Chan *et al* 1991; Flower *et al* 1981, Leone *et al* 1977) and to the lowering of soil oxygen (Flower *et al* 1981).

In this study there was a significant negative linear relationship between methane and oxygen, and between carbon dioxide and oxygen in the soil atmosphere. One explanation for this would be that methane and carbon dioxide displaced oxygen (Chan *et al* 1991, Lan & Wong, 1994, Moffat & Houston, 1991). As in this investigation Chan *et al* (1991) and Lan and Wong, (1994) found a significant positive correlation between methane and carbon dioxide. However, here methane was only detected in the soil when carbon dioxide concentrations were in excess of about 9% and oxygen levels were already depleted to below 7%. The raised carbon dioxide level and reduced oxygen level showed that landfill gas was infiltrating into the soil atmosphere, so therefore one would expect methane to be

detected. This suggests that when oxygen concentrations are high any methane infiltrating into the soil atmosphere is completely oxidised by soil bacteria into carbon dioxide, and so methane is not detected, and carbon dioxide levels increase. However, when oxygen levels are depleted to below about 7%, by this process of oxidation (and displacement by carbon dioxide), there is no longer sufficient oxygen to oxidise all the methane, and therefore, methane concentrations in the surface soil atmosphere become detectable. Therefore these results provide evidence that bacterial oxidation of methane in the landfill soils is also responsible for the consumption of oxygen and thus the lower levels of the oxygen measured (Haarstad, 1997; Hoeks, 1983).

Table 4.18 shows the range of landfill root zone methane, carbon dioxide and oxygen concentrations reported, from field studies, to be responsible for poor plant growth and survival. Methane concentrations ranged between 0.9%- 50% whilst carbon dioxide and oxygen concentrations ranged between 1% - 21%, and 4.7% - 17.8%, respectively. Differential species tolerance and specific additional varying landfill soil conditions (e.g. bulk density, moisture, nutrients etc.) affecting plant susceptibility to landfill gas, may be responsible for the varying results between the different field studies.

The summary of gas concentrations reported to be responsible for poor vegetation growth, indicate that if the soil methane, carbon dioxide and oxygen concentrations, are in excess of 14%, 14% and less than 12%, respectively, then high plant mortality and stunted growth can be expected (Table 4.18). Considering that the carbon dioxide, methane and oxygen concentrations in the cover material on the Bisasar Road landfill (i.e. in the no topsoil plot) were 48.4%, 41.9% and 0.6% respectively, the resultant overall poor tree survival was to be expected.

The much higher concentrations of methane and carbon dioxide measured on the Bisasar Road landfill by comparison to the concentrations measured on other landfills, as summarised in Table 4.18, could be related to the depth of decomposing waste material. The area of the investigation on Bisasar Road landfill had approximately 30m of decomposing waste beneath it, whilst landfills with relatively lower soil gas concentrations had a shallow depth of waste. For example, the Pitsea landfill had maximum depth of 7m (Moffat & Houston, 1991), Edgeboro landfill had 9m of waste (Gilman *et al* 1981), and the Cross Lane landfill had only 3m of waste fill (Wong, 1988), all of which had methane and carbon dioxide concentrations within the range 1 – 18%, which were considerably less than the concentrations measured on the Bisasar Road landfill. Coalgate Lane Landfill had a comparable depth of waste (20m) with the Bisasar Road Landfill, and also had a comparable levels of methane (39-45%) in the soil atmosphere (CO_2 and O_2 were not measured) (Wong, 1988). This reinforced the suggestion of a positive relationship between the depth of the waste and higher landfill gas levels in the soil atmosphere.

Considering the depth of the waste in a landfill site can influence the concentrations of landfill gas in the soil, it would probably also influence the success of vegetation growth. However, the installation of a passive or active gas venting system could help to reduce the concentrations of methane and carbon dioxide in the soil. For example, the Gin Drinkers' Bay Landfill had a greater depth of waste (57m) than the Bisasar Road landfill, however, it had a passive gas venting system installed, resulting in lower maximum methane (17%) and carbon dioxide (18%) concentration measured (Lan & Wong 1994).

Several laboratory investigations used gas concentrations comparable with those on the Bisasar Road landfill. Arthur *et al* (1981) conducted a laboratory experiment in which one

year old *Acer rubrum* (red maple) and *Acer saccharum* (sugar maple) were planted in soil fumigated with 3% O_2, 40% CO_2, 50% CH_4 and 7% N_2 for 48 days. Both species suffered chlorosis and abscission of the lower leaves. Another laboratory experiment conducted by Leonard and Pinckard, (1946) using cotton seedlings found that with oxygen concentrations maintained at 21%, a carbon dioxide concentration of 30-45% reduced root and shoot growth, whilst a carbon dioxide concentration of 60% prevented all root growth and greatly reduced shoot growth. This showed that even under high ambient oxygen concentrations high carbon dioxide levels can effect plant growth. It may be concluded that carbon dioxide in landfill soils has a more important role in determining root growth than oxygen concentration (Chan *et al* 1991). Considering the high level of carbon dioxide found in the root zone of trees on the Bisasar Road landfill the resultant high mortality and reduced growth seen in this experiment was probably primarily due to the soil CO_2 conditions.

The application of a topsoil layer over the original landfill cover material, resulted in a significant reduction in the concentrations of methane and carbon dioxide in the soil atmosphere, but had no significant effect on the low levels of oxygen. Muntoni & Cossu, (1997) found similar results in which a layer of compost reduced landfill gas emissions. In this study, the regression analysis of oxygen versus carbon dioxide (R^2=0.53; $p<0.01$) showed that oxygen in the soil atmosphere was reduced to zero when carbon dioxide concentrations were in excess of 21%. This measured reduction in oxygen with increasing carbon dioxide was probably due to displacement by carbon dioxide and methane and methane oxidation (Barry, 1987; Chan, *et al* 1991; De Rome *et al*, 1997; Dobson & Moffat, 1994). Although the concentrations of carbon dioxide and methane were significantly reduced by the application of topsoil, the levels, 25.6 (±0.7) and 22.3 (±1.3),

respectively, were still sufficiently high to result in very low oxygen conditions, and no significant difference in soil oxygen between the two landfill plots was measured. The levels of carbon dioxide and methane in the topsoil on the landfill were still in excess of concentrations found to be generally associated with high plant mortality and poor growth (Table 4.18).

The ratio of carbon dioxide to methane in the topsoil layer was not significantly different to that in the landfill cover material. Therefore, it is unlikely that the bacterial oxidation of methane into carbon dioxide was taking place at a faster rate in the topsoil layer in comparison to the landfill cover material. If this is the case, then the bacterial oxidation of methane did not account for the significantly reduced concentrations of methane measured in the topsoil plot. The increased physical resistance to the flow of landfill gas presented by the topsoil may have played a part in reducing gas concentrations. Landfill gases tend to flow along paths of least resistance from the decomposing waste to the atmosphere (Flower *et al* 1981). The layer of topsoil could probably, either slow the flow of gas, or change the main direction of flow, thus, reducing the concentrations of methane and carbon dioxide detected in the topsoil layer. Another possible explanation may be that the lower compaction of the topsoil layer in comparison to the landfill cover material resulted in a greater influx of gases from the atmosphere, thus diluting the concentrations of gases in the soil atmosphere. However, neither of these ideas were tested in this present investigation.

The soil temperature on the landfill plots was significantly higher than that on the control and was positively correlated with soil methane and carbon dioxide levels. The exothermic decomposition of waste produces warm gases which can warm the soil as it filters through, therefore the raised soil temperature and correlation with landfill gas concentrations are not

uncommon (Chan *et al* 1991; Gilman *et al* 1981). However, similarly to the finding of Moffat and Houston (1991) the thicker layer of cover material provided by the additional topsoil layer (topsoil plot) resulted in lower soil temperature. This was attributed to the greater distance between the surface soil layers and the underlying heat source (decomposing waste), as well as the significantly lower carbon dioxide and methane levels that were found in the topsoil layer. However the soil temperatures measured on the landfill and the control were within the optimum range for tree growth of 10-30°C (Ruark *et al* 1982), therefore it was unlikely to be a factor resulting in differential tree survival and growth.

From the soil chemical and physical analysis of the conditions on the control and experimental plots K, Mg, pH, conductivity, extractable Mn, soil moisture and stone content were significantly different between the plots. Therefore, of the soil chemical and physical characteristics measured these were the most likely to be responsible for any differences in tree performance between the plots.

The concentrations of K (168.7 mg kg^{-1}) were significantly higher in the landfill cover material (no topsoil plot) by comparison to the topsoil (topsoil plot and control plot). However, deficiencies, and not high concentrations, of K are more likely to cause poor plant growth and survival (Munshower, 1994). Thus, the high concentrations of K in the landfill cover material would probably not have any negative effect on tree performance. Mg concentrations were significantly lower in the landfill cover material by comparison to the topsoil (topsoil plot and control plot). Again, the main concern about Mg is soil deficiencies and not excesses (Munshower, 1994). The soil Mg concentrations (168.3 mg kg^{-1}) in the landfill cover material were within the 'normal' soil range of 40 – 5000 mg kg^{-}

[1] (Grimshaw *et al* 1989), although the concentrations were in the lower part of the 'normal' range.

Moffat and Bending, (1992) and McKendry, (1996) recommended similar soil pH ranges suitable for revegetation of 3.5 – 8.5 and 4.5 – 8, respectively. Therefore, although the pH of the landfill cover material (pH 8.1) was significantly higher than that of the soil used on the topsoil plot and control plot (7.2 - 7.4) it is unlikely to have accounted for the differences in the tree performance observed. However, it is interesting to note that higher soil pH values are often associated with anaerobic conditions since the reduction process removes hydrogen ions from the soil solution thus the pH of acid soils on landfills can rise considerably (Smith, *et al* 1999).

The minimum standard of soil conductivity for woodland establishment on landfills is <2mS cm^{-1} (Moffat and Bending, 1992), however, the conductivity in the landfill cover material (no topsoil plot) was 3.7mS cm^{-1} in this investigation. This higher conductivity in the landfill cover material is often caused by leachate contamination and can have a negative influence on vegetation performance (Dobson & Moffat, 1994; Tong & Wong, 1984). The raised pH and the significantly higher concentration of K in the cover material, as found by Winant *et al,* (1981) in leachate irrigated soils, reinforced the evidence of leachate contamination of the landfill cover material (no topsoil plot). This indicated that further leachate related variables, not measured in this investigation, such as depressed soil solution osmotic potential (Cureton *et al* 1991), increased sulphate, sodium, chloride and metals in the soil (Ettala, 1988), which can have negative effects on vegetation, may have influenced the trees growth and survival. The conductivity of the topsoil placed on the

landfill was not significantly different to that on the control and below the threshold standard recommended by Moffat and Bending, (1992).

The measured concentrations of extractable Mn on the experimental plots showed that the available Mn concentrations in the topsoil placed on the landfill (topsoil plot) had significantly increased (six fold), to levels which were not significantly different from those measured in the landfill cover material (no topsoil plot). There were two possible explanations. The first being the contamination of the topsoil with leachate after it had been instated onto landfill and the second could be the anaerobic soil conditions increasing the proportion of available soil Mn.

Leachate irrigation or unwanted contamination of landfill soils has been shown to significantly increase the concentrations of soil Mn (Tong & Wong 1984; Winant *et al* 1981). The pH, K concentration and conductivity measurements in the landfill cover material may indicate leachate contamination, and it is possible that the high levels of Mn in the landfill cover material and the increase in Mn levels in the topsoil may be related to leachate contamination of the soil. However, the analysis of the total metal content of the soil clearly showed that there was no difference in Mn levels between the plots indicating that there was no external loading of the topsoil by leachate contamination. In fact the landfill cover material generally had lower total metal content than the topsoil used in the experiment. Thus the higher Mn levels were more likely due to the anaerobic soil conditions.

Under aerobic soil conditions manganese occurs as the manganic ion Mn^{4+} which has a limited solubility and may be oxidised to form an insoluble, precipitable oxide (Crawford,

1989; Menser *et al* 1979). However, the reducing conditions of anaerobic soils result in the highly soluble manganous ion Mn^{2+} (Crawford, 1989; Menser, *et al* 1979; Munshower 1994). Therefore, the anaerobic soil conditions on the landfill plots probably resulted in the formation of the highly soluble Mn^{2+}. This is of significance as high Mn concentrations can be phytotoxic to many sensitive species. High available manganese concentrations induce iron chlorosis, and also cause brown necrotic spots on plant leaves, due to antagonism between these ions for uptake by the roots (Crawford, 1989). Mn is not toxic if the soil pH is greater than 5.5 because the manganese solubility is reduced with increasing soil pH (Munshower, 1994; Winant *et al* 1981). Therefore, it may be argued that in a soil with a pH value of 7.4 (topsoil plot) and 8.1 (no topsoil plot) available Mn concentrations should be very low. However, the anaerobic soil conditions on the landfill plots may have formed strong reducing conditions. Thus, landfill soils may be a relatively unique situation with high pH and strongly reducing conditions. However, it must be noted that the sampling of the soil would have removed the reducing conditions, however, the measured extractable Mn levels were still very much higher. This would suggest that the anaerobic conditions changed the ratio between total and extractable Mn, which was maintained even after the anaerobic conditions had been removed (i.e.on air drying the sampled soil before analysis). It can be concluded that the high level of available Mn in the topsoil and cover material of the landfill, in conjunction with the anaerobic conditions may have influenced the growth and survival of the trees.

The landfill cover material had significantly lower soil moisture, as found by Gilman *et al* (1981) in a similar experiment. Highly compacted soils usually have reduced soil hydraulic conductivity and volumetric water content (Ruark *et al* 1982; Taylor & Brar 1991), possibly explaining the lower moisture content in the landfill cover material, which are

characteristically compacted (Dobson & Moffat, 1994). The application of topsoil over the cover material on the landfill, and so increasing the soil depth, significantly improved the moisture levels on the landfill, as found by Moffat & Houston, (1991). However, the moisture levels in the topsoil plot were still significantly lower than the control plot. Considering that species tolerant to flooded soils (i.e. soils with high water content) have shown tolerance to high landfill gas conditions (Arthur *et al,* 1981), the low moisture conditions on the landfill may present a problem for such plant species.

Considering that all the plots were sufficiently close to each other as to receive the same rainfall, a possible explanation for moisture differences may be the physical structure of the waste cover material. The structure of the landfill cover material below the topsoil on the landfill may prevent the upward migration of soil moisture into the topsoil during dry periods and reduce infiltration of rain water into the plot without topsoil, thus possibly accounting for the different moisture conditions. A possible explanation for the higher moisture levels in the control plot may be the underlying weathered dolerite silty clay. This may allow for the upward migration of moisture during dry periods, unlike the cover material on the landfill. The significant difference ($p<0.01$) in stone content on the landfill plot by comparison to those receiving topsoil may help to confirm the suggestion that landfill soil structure reduces upward capillary movement of water. Thus, depending on the size of the stones, the continuity of the soil pore space maybe lacking, thus preventing upward migration of moisture by capillary action. Similarly, the 'cementing' together of the stones by the increased compaction possibly results in decreased rainfall infiltration.

The stone content of soil is measured in different ways by different researchers. It is measured by volume, and / or weight and the size of the particles defined as stones differs

considerably. This makes it difficult to compare these results with other research conducted. However, it can be concluded that lower stone contents are usually preferred for landfill restoration (McKendry 1996; Moffat & Bending 1992). Therefore, the application of topsoil with a significantly ($p<0.01$) lower stone content, over the landfill cover material, should have resulted in improved conditions for tree root growth.

In summary the key environmental variables in the landfill cover material which were most likely to influence the growth and survival of the trees would have been: the high carbon dioxide, and low oxygen concentrations in the soil atmosphere, high conductivity, high extractable Mn concentrations, low soil moisture and high stone content and possibly low magnesium concentrations. The application of topsoil was able to reduce the severity of carbon dioxide concentrations, although it still remained within the range of concentrations at which poor plant growth and survival could be expected. The topsoil also had a better moisture content, stone content, conductivity and level of Mg. However, the Mn concentrations were just as high as the landfill cover material suggesting that topsoil quality can deteriorate with time when used on landfills.

CHAPTER 5: TREE GROWTH AND SURVIVAL: A SOIL FUMIGATION EXPERIMENT

5.1 INTRODUCTION

The field-based research has provided information about the range of environmental conditions on the landfill and the varying response of different tree species (Chapters 3 & 4). However, the heterogeneity and dynamic nature of the landfill environment and the high mortality of less 'tolerant' species made it difficult to identify key plant characteristics, which could explain differential species performance on the landfill. It was also difficult to establish the relative importance of high CO_2 and low O_2 concentrations in the soil and the potential for antagonistic, additive or synergistic effects between these two variables.

To provide an experimental approach, a soil fumigation system was designed, constructed and established. Using bottled gas, the experimental apparatus was capable of mixing carbon dioxide and oxygen into four different ratios, thus supplying four different gas regimes. A combination of automated low pressure mixing of gases and a pulse flow fumigation technique made the experimental apparatus uniquely economical to operate, thus allowing for relatively longer fumigation periods to be achieved. The apparatus was used to fumigate the soil of 80 potted landfill 'tolerant' (*Barringtonia racemosa*) and 'non-tolerant' (*Harpephyllum caffrum*) trees. The 4 gas regimes (treatments) used were the following: "normal" soil O_2 and CO_2; high CO_2 and normal O_2; low O_2 and normal CO_2; high CO_2 and low O_2.

Using the fumigation system, the hypothesis that one species (*Barringtonia racemosa*) was significantly more tolerant than the other (*Harpephyllum caffrum*) was tested. Measurements were made of the above and below ground growth and functional plant morphological, anatomical, and physiological characteristics to evaluate the responses of the two species to the 4 soil gas treatments.

5.2 THE SOIL FUMIGATION SYSTEM

5.2.1 Design objectives

A fumigation system consists of an apparatus, which provides gases at known concentrations to chambers in which plants can be exposed. The usual fumigation system provides gases into the chamber which change the gas mixture of the atmosphere around plant shoots with the plants rooted in pots of soil. A soil fumigation system must change the gas concentrations in the soil atmosphere, thus the chambers are containers in which plant roots and not shoots are exposed. Most fumigation experiments have focused on atmospheric gases and relatively little research has been done on the soil atmosphere and its effect on plants. The major focus of most soil atmosphere research has been on the effects of waterlogging on plants and the effects of low soil oxygen and high carbon dioxide. Most researchers, such as Bacanamwo and Purcell (1999); He, *et al* (1999), and Loreti and Osterheld (1996) induce low oxygen conditions by the actual flooding of the soil. However, this technique does not allow for the easy manipulation of the actual oxygen or carbon dioxide concentrations i.e. to predetermined or desired levels. In order to achieve this some researches such as Huang *et al* (1997), Moog and Bruggemann (1998), and Voesenek *et al* (1999) preferred using hydroponic solutions that could be flushed with the required concentration of oxygen, carbon dioxide and nitrogen. However, these experimental methods do not create an experimental environment common to the usually

low oxygen, high carbon dioxide, and *dry* soil of a landfill. Therefore, although similarities in plant responses to waterlogging and landfill conditions have been observed by Arthur (1981), Barry (1987) and Chan *et al* (1991), and waterlogging research provides insight into potential plant responses, none of the experimental systems used and described to date could be directly adapted to the needs of this experiment.

Of primary design importance for this experiment was complete control of the gas concentrations in the fumigation chambers otherwise the system would not be an improvement relative to field experiments. Secondly, in order to assess the response and adaptation of relatively large and slow growing tree saplings, the system had to fumigate large volumes of soil for a long period of time. Based on the conclusions from the field experiment (Chapter 4) the within species variability and duration before health affects were observed, an experimental period of 140 days and at least 10 replicates per species per treatment were deemed necessary. Each tree would require its own chamber so as to increase the validity of the replication, especially to avoid pseudoreplication, thus a total of 80 identical chambers were required (2 species x 4 treatments x 10 replicates).

Research relating directly to the effects of landfill gas on plants has been mostly field-based and suffers the usual high level of environmental heterogeneity which often makes it difficult to draw definitive conclusions. There have been relatively few studies that involve the simulation of landfill soil gas conditions. Chan *et al* (1991; 1998) described a fumigation system that used simulated landfill gas generated through the anaerobic digestion of pig manure. The largest of their experiments consisted of a single simulated landfill gas treatment with 10 replicate pots in which the roots of 10 species of tree were fumigated for 42 days. The gas diffused from the bottom of the pot through the soil and

flowed freely from the soil surface into the surrounding atmosphere. The gas concentrations measured within the pots were highly variable and there was no direct control of the gas composition. Due to the free flow of gas from the soil surface into the atmosphere, the contamination of the air around the shoots with the simulated landfill gas, could also make the interpretation of plant response to root fumigation difficult. The experimental period was only one third of the duration required for this experiment and the reliability of anaerobic digesters as a gas supply was a concern. The use of a single pot / fumigation chamber for 10 trees also raised concern for the validity of the replication and if root responses of individual plants could be measured. Thus the fumigation system described by Chan *et al* (1991) was not suitable for the needs of this experiment.

The fumigation system used by Arthur *et al* (1981) was very similar to Chan *et al* (1991), but used only two 88 L garbage cans in which the roots of two species of maple were fumigated for 50 days. Unfortunately, this design also lacked the scale and level of replication needed for this experiment. However, it did make use of a cylinder of pre-mixed gas instead of anaerobic digestion as a simulated landfill gas supply. However, the gas concentrations within the soil still showed high levels of fluctuation even with a more reliable gas supply. Arthur *et al* attributed the high level of fluctuation to the variability in atmospheric conditions. This highlighted the need for control of the atmospheric influence on the soil gas concentrations, and suggested that the free flow of gas from the soil surface into the surrounding atmosphere was probably not a suitable approach.

Marchiol *et al* (1999) described a 12-day experiment assessing seed germination of 4 different ground covers in three replicate atmospheres of simulated landfill gas. The fumigation chambers were sealed boxes with gas flow output pipes that prevented over

pressurisation but allowed for a positive gas pressure to be maintained within the chamber. This reduced possible atmospheric influence on the gas concentrations in the chamber. The system also made use of bottled gas that was commercially pre-mixed to the desired oxygen and carbon dioxide concentrations. The result was better control of gas concentrations within the chambers. The use of a pre-mixed gas and a slightly pressurised chamber was a suitable solution for control of gas composition. However, the scale of Marchiol's design was significantly smaller in comparison to the needs of this experiment, and when scaled-up the cost of premixed gas could be prohibitive, further, the chamber design was not suitable for the fumigation of tree roots. Marchiol *et al* did use an interesting device for the splitting of the gas supply into equal gas flows for each of his 12 chambers. The device operated using two simple principles. The first was the total diameter of the gas output pipes should be less than the diameter of the gas distribution cylinder, and the second was that sufficient gas, in terms of pressure, was available in the distribution cylinder to supply all the chambers (*pers com* Marchiol, 1999). However, the design would need marked adaptation in order to handle 80, much larger, chambers instead of 12.

The work by Zhang *et al* (1995) also made some interesting design contributions. They were interested in the fumigation of tree roots with simulated landfill gas for investigating the response of nitrogen fixing root nodules on two leguminous species. Zhang *et al* made use of bottled gas but used separate cylinders of nitrogen, carbon dioxide and oxygen and mixed the gases by setting the flow rates of the three component gases according to the treatment requirements. This was a more economical way of achieving complete control of the gas concentrations than commercially premixed gas. Again the scale of the experiment was relatively small, with only 32 chambers that were fumigated for 8 days. There were

some further interesting design features that were worth noting. The roots of the trees were fumigated by placing the pots into plastic bags, with inlet and outlet pipes attached, and the bag was sealed around the base of the stem ensuring fumigation of the root material only. The gas outlet from the bag was a small diameter pipe that was submerged in water, thus allowing for a positive pressure to develop inside the bag and preventing the influence of external atmospheric conditions. Although, the use of a plastic bag is probably not robust enough for an experiment of 140 days the idea of using a semi-closed fumigation system appeared to be a sensible design feature. Zhang *et al* (1995) also used an interesting technique for ensuring equal distribution of gases between the 8 pots within each treatment. This was done by fumigating each pot in sequence, controlled by a timer and eight solenoid valves. Although this was an innovative approach the cost of a solenoid valve for every chamber was regarded as too expensive.

This review of the other landfill gas fumigation experiments provided some interesting design features that have been mentioned. However, in terms of the aims of this experiment as well as the number of treatments, number of trees and the duration needed for this experiment, it appears to be unique and no single fumigation apparatus designed to date fits the exact requirements. Thus in order to achieve the goals of this experiment a combination of the ideas used in previous fumigation systems and some new ideas were required. These are discussed in the next section.

In terms of the fumigation treatments, it is generally agreed that the direct effect of soil methane on plants is minimal relative to low oxygen and high carbon dioxide concentrations (Dobson & Moffat, 1994; Leone *et al* 1977; Spreull & Cullum, 1987). In this investigation, the chapter on grasses concluded that elevated carbon dioxide levels in

the soil were the greatest factor limiting grass growth and methane had very little direct effect (Chapter 2). A similar conclusion was made by Chan *et al* (1991) and Wong and Yu (1989) who observed a greater negative correlation between soil carbon dioxide concentrations and vegetation cover relative to that of methane. Methane appears to have no direct toxicity to plants and concentrations as high as 60% have been shown to have no phytotoxic effect (Flower, *et al* 1981). In fact methane cannot be metabolised by plants and has been used as a tracer gas in transpiration studies (Morris & Dacey, 1984). Thus, the experimental focus taken in here was on the effects of oxygen and carbon dioxide only and used inert nitrogen as a carrier gas.

In order to get a detailed assessment of the plants response to the treatments it was important that the plants were growing and not killed by too severe treatment conditions. Using the gas-depth profile in conjunction with rooting depth and tree mortality from the previous field work and a literature survey of measured landfill soil gas concentrations (Table 3.10), a carbon dioxide concentration of 25% and oxygen concentration of 3% were considered suitable thresholds for the fumigation experiment. In order to investigate the effects of these gases the following 4 treatments and mixed soil gas concentrations were used: normal soil O_2 (20%) and normal CO_2 (2%); high CO_2 (25%) and normal O_2 (20%); low O_2 (3%) and normal CO_2 (2%); high CO_2 (25%) and low O_2 (3%).

5.2.2 Design

A schematic diagram of the fumigation system design is provided in Figure 5.1 with the details and the reasons for particular design choices provided below. Gases supplied in high-pressure cylinders are a common source used for fumigation systems, however, it is more expensive than gas produced through anaerobic digestion, but the quality is far more reliable. The use of a commercially premixed gas regime in a single cylinder is convenient and ensures accurate control of the gas concentrations, however, for an experiment of this size and duration the cost of premixing was prohibitive. Separate gas cylinders of gas that can be used to mix the required gas regimes at low pressure was a more economical approach. Technical grade carbon dioxide, oxygen and nitrogen were purchased from Afrox (Pty Ltd) and supplied separately in the largest available high-pressure cylinders, 31.3kg (15300kpa), 11.5kg (17500kpa) and 11kg (20000kpa), respectively. In order to prevent system failure due to lack of gas, a number of cylinders for each gas were connected by a high-pressure manifold. The manifolds for the three gases were constructed out of appropriate cylinder adapters and Parflex 27579kpa pressure rated flexible piping, and connected 5 cylinders of nitrogen, 4 carbon dioxide and 2 oxygen into three separate manifolds (Figure 5.2 and 5.3).

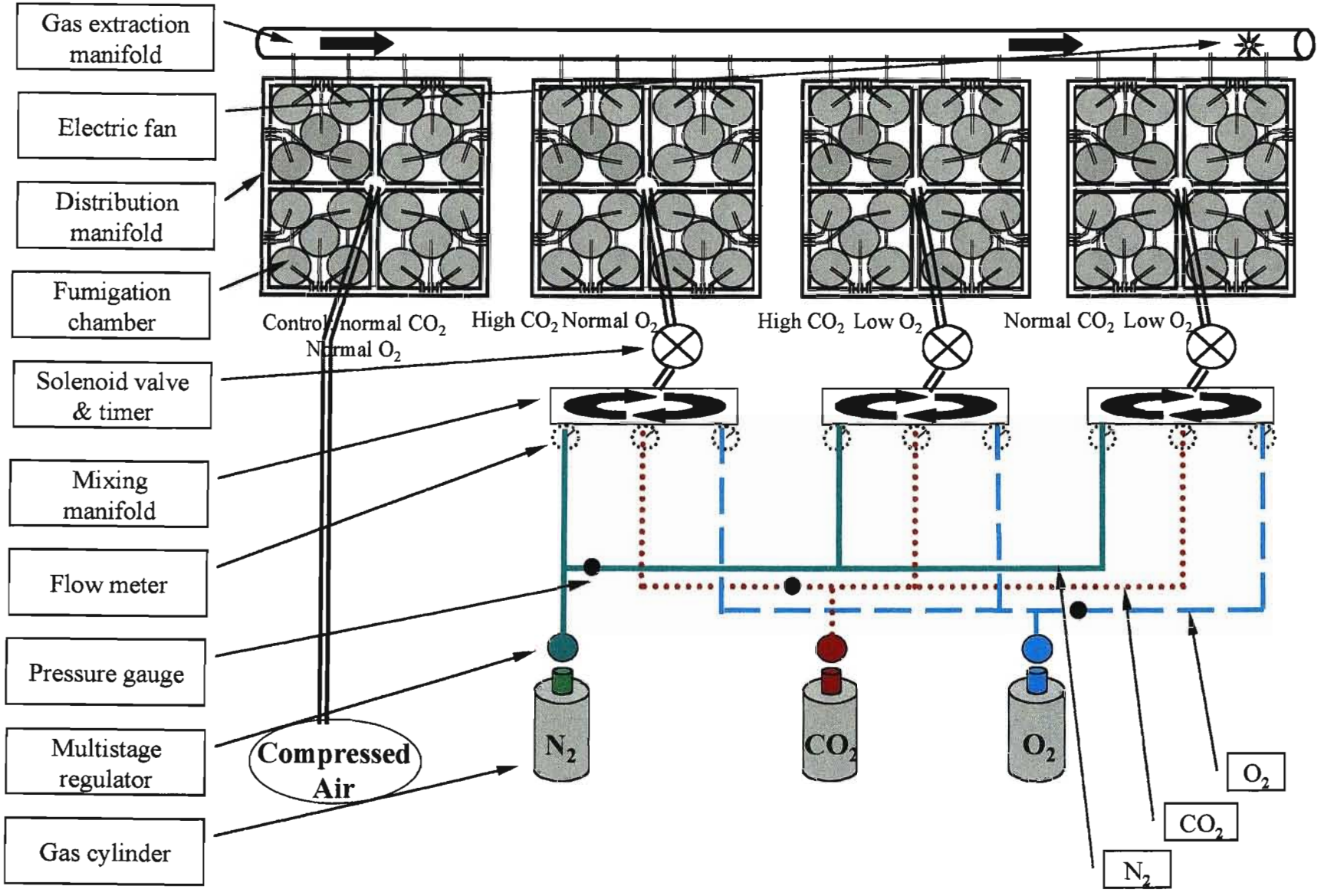


Figure 5.1 :Schematic diagram of soil fumigation system

To achieve the desired soil atmosphere conditions for the control, compressed air supplied by an electric compressor was more economical than commercially supplied cylinders of compressed air. The air supply from the compressor was passed through an oil trap, to prevent contamination, and the pressure regulated to 200kpa.

Three separate multistage regulators, also adjusted to a constant output pressure of 200kpa, controlled the gas supply, from the high-pressure manifolds, needed for the gas treatments. The gas from each regulator was connected with 6mm poly-natural nylon pressure tubing and divided, using 6mm brass elbows and T-junctions, into 3 separate gas outlets to be used for each treatment. It was critical for the mixing of the gases that there was sufficient volume of gas within the poly-natural tubing to supply the demand of the three treatments at the same time and without a pressure gradient developing. The 200kpa supply pressure was calculated as sufficient, however, a set of pressure gauges were installed in-line between the regulators and the mixing manifolds to monitor pressure conditions within the supply line during operation (Figure 5.4).

The mixing of the gases into the appropriate gas ratios was achieved using Dwyer Visi-Float flow meters and specially designed gas-mixing manifolds. Each treatment required a set of three flow meters and a mixing manifold to achieve the required gas ratios (Figure 5.5). The control did not require any special mixing of gases, thus the air supply was connected directly to a single flow meter without a mixing manifold. In order to achieve the desired gas ratios it was important to note that the actual quantity of gas (molecules) measured using a flow meter varies with the specific gravity (SG) and the pressure of the gas used. Thus it was important to convert the observed flow meter reading into an actual gas flow which was pressure and SG corrected using the following equations.

Pressure correction

$$Q_2 = Q_1 \times \sqrt{\frac{P_2}{P_1}}$$

Q_1 = Observed flow meter reading

Q_2 = Actual flow corrected for pressure

P_1 = Standard atmospheric pressure, 14.7 PSI

P_2= Actual Pressure, 14.7 PSI + pressure inside flow meter

Specific gravity correction

$$S_2 = S_1 \times \sqrt{\frac{1}{S.G.}}$$

S_1 = Observed flow meter reading

S_2 = Actual flow corrected for specific gravity

1 = Specific gravity of air

S.G. = Specific gravity of gas being used in flow meter originally calibrated for air

Using the above equations the flow meters were adjusted to provide the required ratio of each gas flowing into the mixing manifolds. The manifold was a thick wall PVC chamber 100mm in diameter and 500mm long (4 l liquid volume) with the appropriate fittings for the flow meters and the outflow of the mixed gas attached by plastic welding (Figure 5.6). Although the operating pressure of the gas entering the chamber was 200kpa the chamber was designed for a pressure of at least 300kpa, so as to ensure safety.

A 220 Volt solenoid valve on the outflow of the mixing manifold controlled the flow of the three gases through the flow meters (Figure 5.7). When the valve was closed the mixing manifold would fill up to a pressure of 200kpa with the correct ratio of the three gases, as controlled by the flow meters. The opening of the solenoid valve released a pulse of 200kpa mixed gas into the gas distribution manifold and started the cycle of mixing a new batch of gas within the mixing manifold. The opening and closing of the solenoid valves for each treatment was synchronised and automatically controlled by a series of electronic relays connected to a timer (Figure 5.8).

The reason for using a pulse gas flow rather than a continual flow was the first step to dealing with one of the largest design challenges, achieving equal gas distribution between the twenty separate chambers within each treatment. It was critical to have sufficient pressure within the distribution manifold so as to provide all the chambers with equal volumes of gas without a pressure gradient in the manifold developing. However, maintaining a high pressure in an open system is wasteful of gas and costly, and using low pressure is more economical but results in poor distribution. Thus, the use of a pulse flow system allowed a relatively high pressure to be maintained during pulses, thus improving gas distribution but minimising gas wastage. However, the design of the distribution manifold was also critical in terms of reducing the volumes of gas required.

The distribution manifold needed to be small in volume and have 20 small outlets so, even as an open system, it could be easily pressurised by a relatively high volume pulse from the mixing manifold. A simple linear distribution manifold with the inlet on one side proved to be inefficient and required higher than 200kpa to ensure good distribution. This lead to the experimentation with a number of different designs and the distribution was assessed by

the pressure required to attain even distribution. The best approach was a manifold constructed from 16mm diameter PVC piping (Figure 5.9). A single supply pipe was divided into 4 branches that were connected to the four quarters of a continuous ring of 16mm pipe onto which the outlets were evenly spaced. This design ensured that any single outlet would receive gas from two directions simultaneously, thus reducing the development of a pressure gradient within the manifold even at a relatively low pulse pressure of 200kpa. The distribution manifold was also designed as 35% of the total volume of the mixing manifold and the outlet diameters were restricted to 4mm diameter thus ensuring good pressure development within the manifold with each pulse even though it was an open system.

The distribution manifold outlets were fitted with 4mm push-clip pneumatic hose attachments (FESTO, Pty Ltd), although expensive they proved to be very useful because they allowed for easy attachment and detachment of chambers without having to worry about gas leaks. The fumigation chamber feed pipes were made of thick wall 6mm poly-natural nylon tubing that plugged directly into the FESTO clips. It was critical that the feed pipes were all the same lengths and had no kinks, ensuring equal gas resistance and even gas distribution.

The fumigation chambers were made of 20 l polypropylene buckets with lids that seal (Figure 5.10). Gases did not diffuse directly from the surface of the soil into the atmosphere but were ducted away from the foliage of the plants. The chamber was closed off around the stem of the trees, ensuring only the fumigation of the roots. Also this was important for creating back pressure within the chambers allowing better fumigation of the soil, better gas distribution between chambers and better gas use efficiency. It was also

important that the atmosphere surrounding the plants was not contaminated with the fumigation gases as this could effect plant response and create potentially hazardous working conditions. The tree stem was inserted through a 50mm diameter hole in the centre of each lid, using a split cut through half the lid so as not to damage the foliage. Once the lid was placed onto the chamber the split and hole surrounding the stem were sealed using silicon and Genkem foam sealant. These products were found to have sufficient flex so as not to restrict stem growth but still maintained a good seal.

A 60mm screw cap was inserted into the lid of the chambers providing easy access for watering and monitoring the condition of the soil. The gas inlet, gas sampler and gas outlet, which were made of poly-natural tubing, were inserted into the chamber through three separate 6mm holes in the lid (Figure 5.10). The gas inlet tube was glued to the inside of the chamber and the gas was diffused by a 50mm cylindrical air-stone attached to the centre of the base of the chamber. The bottom 5cm of each chamber was filled with 7mm stone to assist with even gas distribution and water drainage in the soil. A polypropylene tap was plastic welded onto the base of each chamber allowing for excess water to be drained if needed. The gas sampler consisted of a 50mm air-stone that was placed into the centre of the potting medium just below the root ball of each tree. After passing through the lid, the sampler tube was sealed with a 6mm plastic plug, which could be removed when a gas sample from the chamber was drawn. The gas outlet was fixed just below the lid of the chamber and allowed for the flow of the gas from the chamber into the gas removal manifold.

Equal lengths of poly-natural tubing were used to remove the gas from the top of the chambers into a 110mm diameter, 7m long PVC pipe. The PVC pipe was fitted with a

small electric fan so as to maintain a very slight negative pressure within the manifold preventing any back flow of atmospheric air into the system. The manifold transported the waste gas out of the greenhouse in which the experiment was conducted.

The fumigation system was in a fully air-conditioned greenhouse with temperature and humidity control. The day / night temperature was controlled to one degree accuracy at 24°C and 20°C respectively, and the relative humidity was kept constant at 50%. As a precautionary measure a temperature-activated alarm was designed and installed, which provided a warning if the temperature varied more than 5°C from the target value. Minimum and maximum temperatures within the greenhouse was checked daily so as to ensure that the air-conditioning system was working properly and the desired conditions were maintained.

Gaseous carbon dioxide is a 'denser-than-air' asphyxiant. A concentration of 10% (100 000ppm) can produce unconsciousness and death, lower concentrations can cause headaches, sweating, rapid breathing, mental depression, visual disturbance and shaking (Mallinger, 1996). Large quantities of bottled carbon dioxide were present within the greenhouse and although an exhaust gas removal system was operational, a precautionary gas leak warning system was important. An electric air pump positioned on the floor of the greenhouse continuously circulated greenhouse air through a clear container of bromothymol blue solution. This blue indicator solution turns yellow in the presence of carbonic acid, which would form if the air bubbling through contained elevated levels of CO_2. The solution could be seen from outside the greenhouse, thus providing a clear

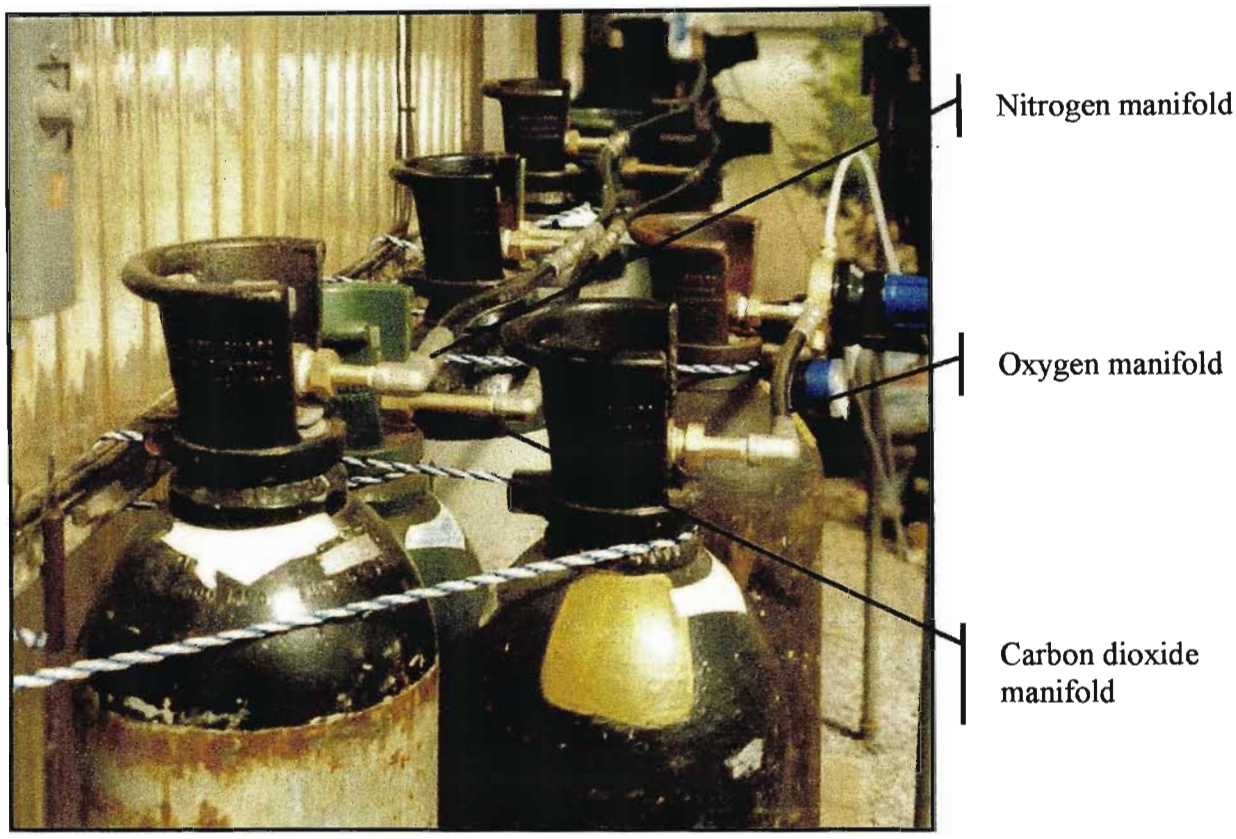


Figure 5.2: A set of cylinders of nitrogen, oxygen or carbon dioxide were connected by a high pressure manifold so as to supply a reliable gas source for the fumigation system.

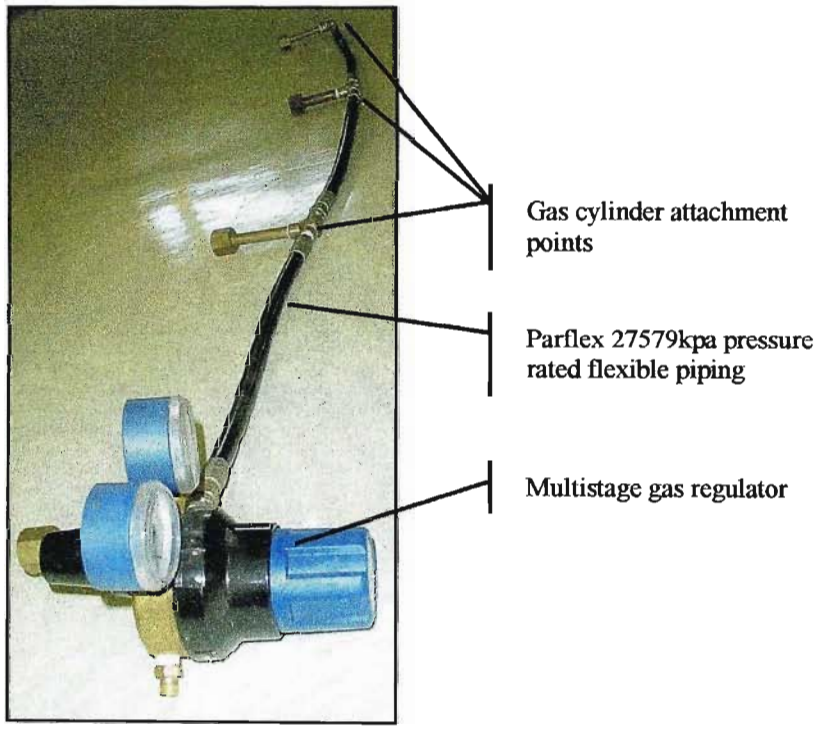


Figure 5.3: Example of a high-pressure manifold disconnected from the cylinders, showing multistage regulator and gas cylinder attachment points.

Figure 5.4: In-line pressure gauges installed in the N_2, CO_2 and O_2 gas supply tubes in order to ensure a 200Kpa supply pressure to the mixing manifold was maintained.

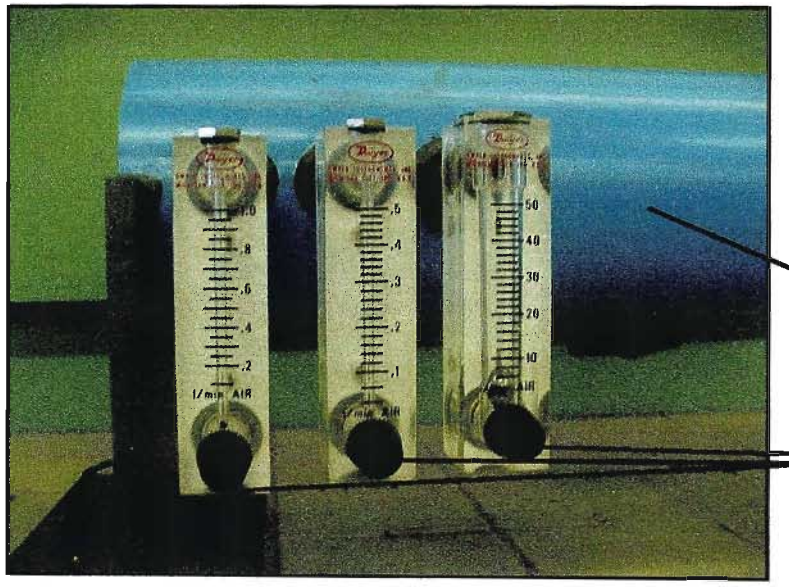


Figure 5.5: The ratio of N2 ; CO2 and O2 in the mixing manifold for each treatment was controlled by three separate flow meters.

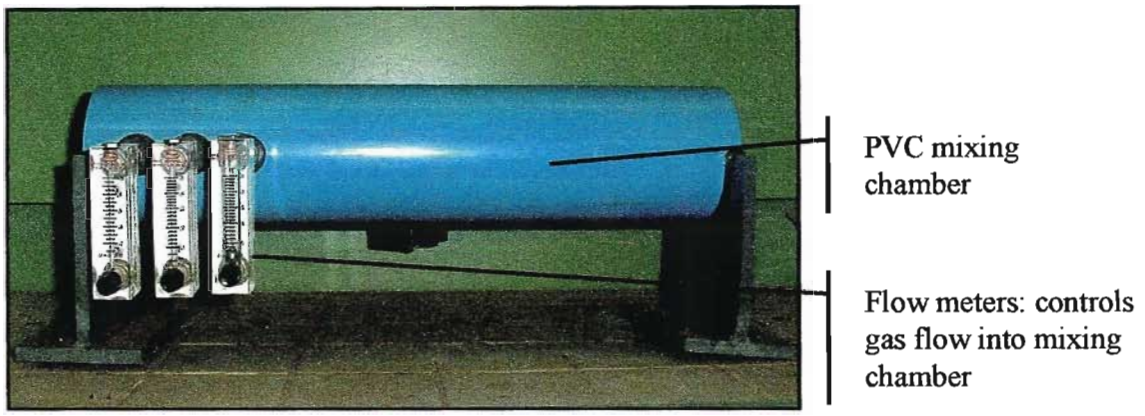


Figure 5.6: Mixing manifold made of thick wall PVC which filled up with the three gases in the appropriate ratio for the treatment as controlled by the flow meters.

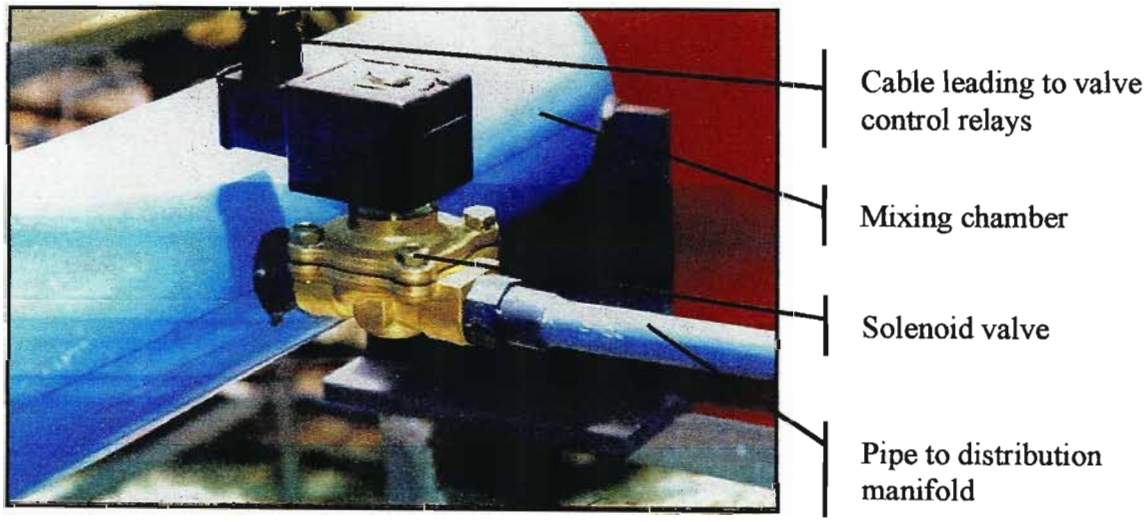


Figure 5.7: Solenoid valve on the outflow of the mixing manifold allows for a pulse gas flow to the distribution manifold to be achieved.

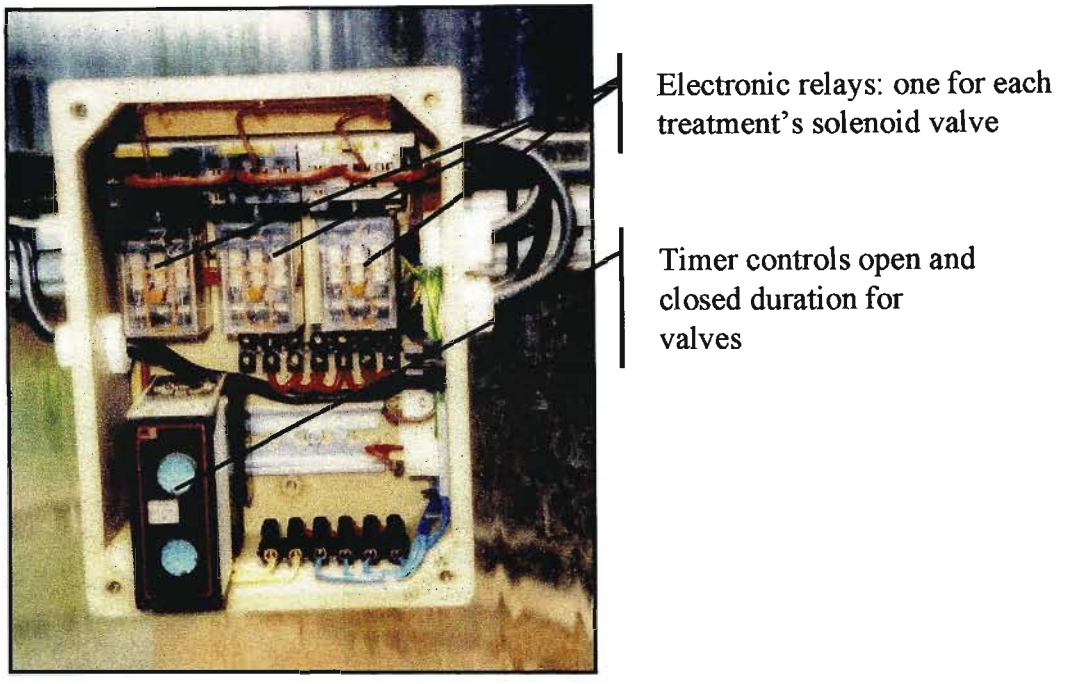


Figure 5.8: Electronic relays and timer used to control the synchronised opening and closing of the solenoid valves for each treatment.

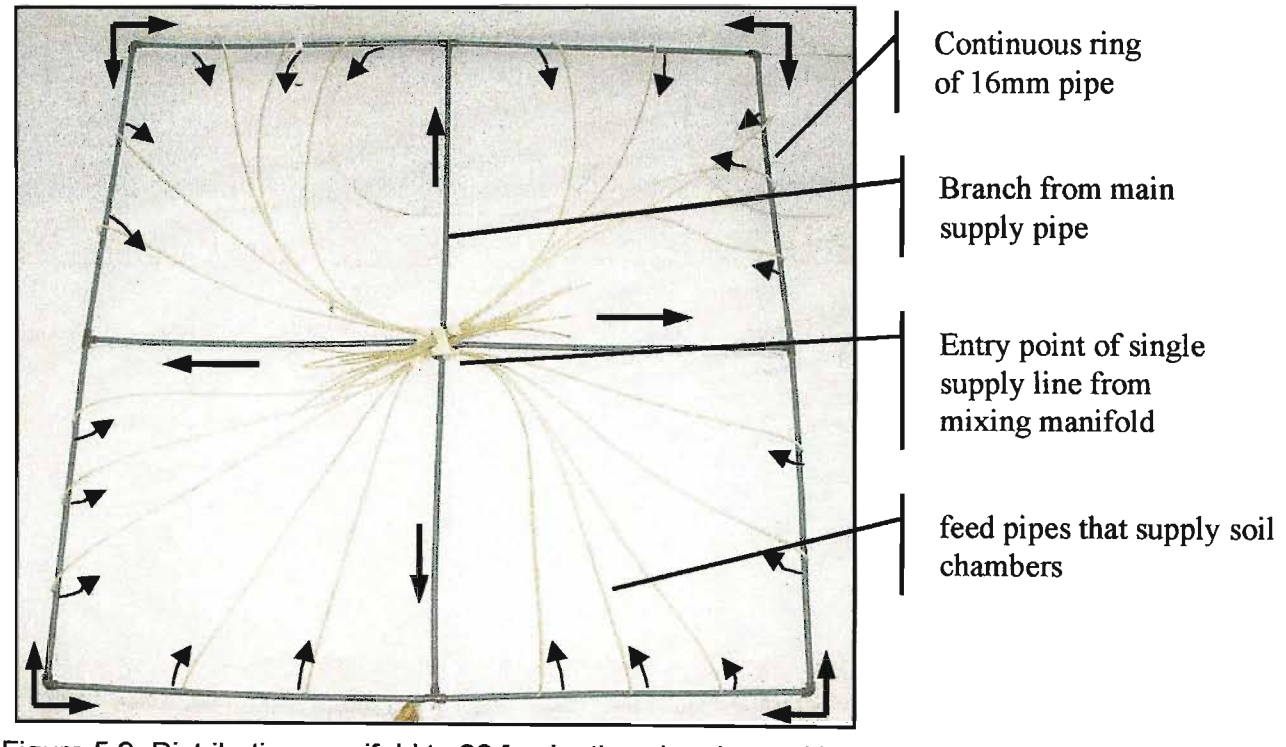


Figure 5.9: Distribution manifold to 20 fumigation chambers with arrows showing direction of gas flow

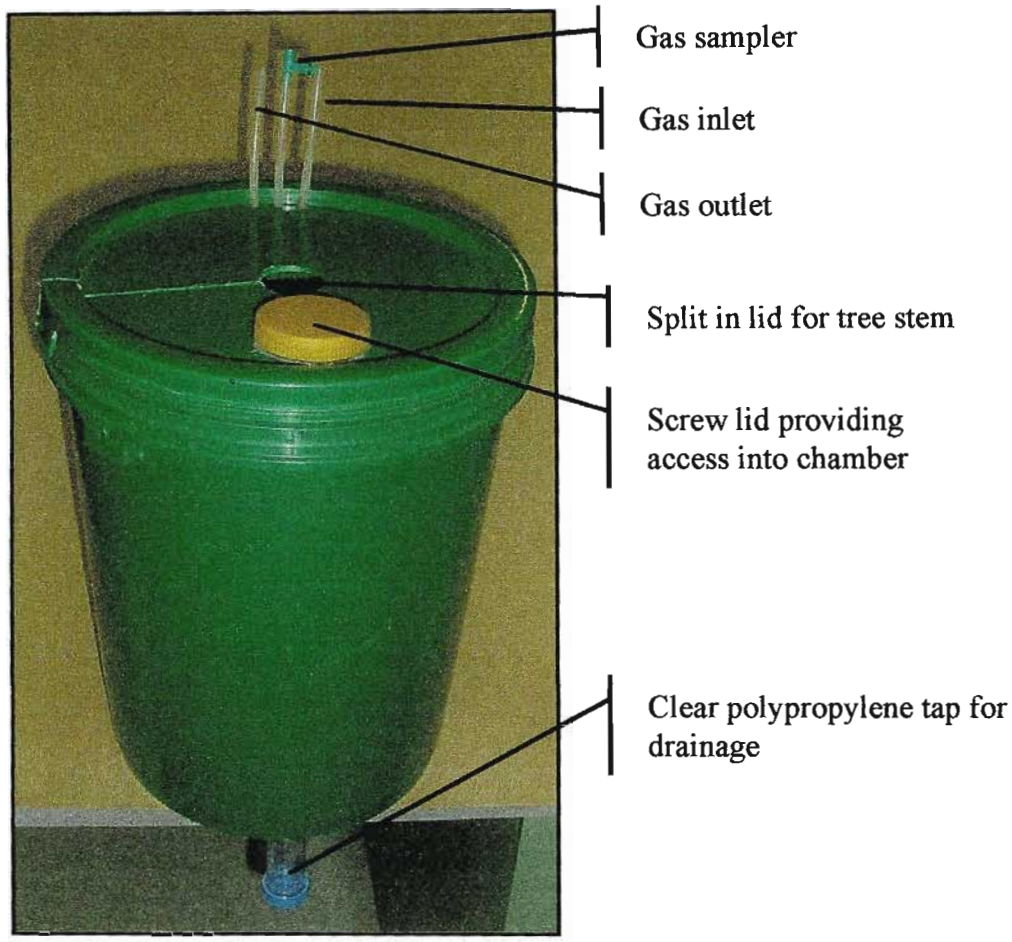


Figure 5.10: Fumigation chamber constructed from a 20l polypropylene bucket

indication of a CO_2 hazard before entering. The levels of oxygen and carbon dioxide within the greenhouse were also checked daily for the first week and then weekly for the rest of the experimental duration using an infra red gas analyser (Geotechnical Instruments GA 94 Infra– Red Gas Analyser).

5.2.3 System evaluation

The flow meters were set according to the theoretical flow rates required to achieve the different gas treatments and the mixed gas flow was checked using a Geotechnical Instruments GA 94 Infra– Red Gas Analyser. During calibration the actual concentrations of CO_2 and O_2 after mixing did not vary more than 3% from the theoretical values calculated from the flow meters, illustrating the efficiency of the mixing manifold.

Initially the solenoid valves were kept open until the air in the chambers was displaced, the valves were then closed and the concentration of gases in the soil within the chambers was carefully monitored. This procedure was done 10 times to calculate the valve closed time and open time that was optimal for maintaining a consistent gas concentration in the chambers without gas wastage. This was subsequently set to 20 minutes closed, 18 seconds open.

The gas concentration within each chamber was monitored 4 times a day during the first 3 days to ensure stability of the gas flow. Thereafter the gas concentration within each chamber was measured every 7 days, using the infra– red gas analyser, to ensure the system was working satisfactorily (n=22). The desired soil atmosphere treatments were achieved (Figure 5.11). The 'high' values, 'low' values or the 'normal' values between the

treatments for either of the individual gases, carbon dioxide and oxygen, were not significantly ($p>0.05$) different. However, the 'normal' values were significantly ($p<0.05$) different from the 'low' and 'high' values within and between the treatments.

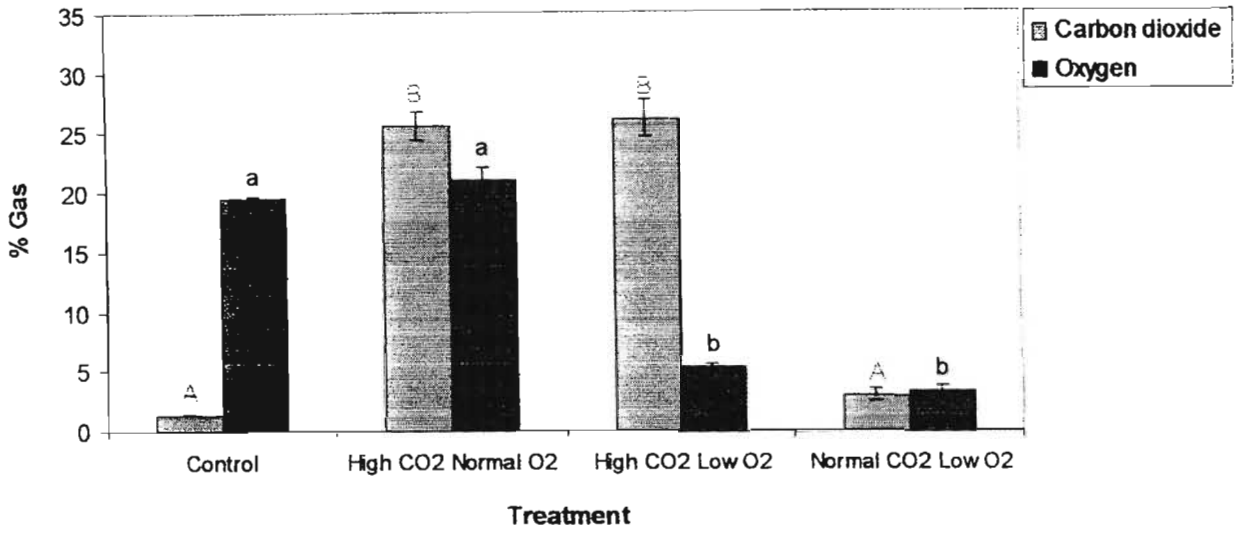


Figure 5.11: Mean (n=22) percentage carbon dioxide and oxygen in the soil of the chambers for each treatment. Significant differences ($p<0.001$) between gas levels between treatments are shown by a change in letters (upper case letters for CO_2, lower case for O_2).

Relatively small variations in the gas concentrations were measured between the chambers within each treatment (Table 5.1), indicating a satisfactory distribution of the mixed gas between the 20 chambers. The waste gas extraction system also worked satisfactorily, as no increase in carbon dioxide levels in the greenhouse were detected and levels remained at approximately 394 $\mu mol\ mol^{-1}$ throughout the experiment, as measured with a LI-COR Portable Photosynthesis System -LI-6400.

Table 5.1: The mean and the 95 percent confidence limit of carbon dioxide and oxygen concentrations measured between the chambers within the treatments during the experimental period (20 chambers per treatment measured 22 times).

Treatment	Carbon dioxide		Oxygen	
	Mean[1]	95% confidence limit	Mean	95% confidence limit
Control	1.38	0.28	19.53	0.27
High CO_2 Low O_2	26.40	1.56	5.17	0.94
High CO_2 Norm. O_2	25.56	1.14	20.97	0.23
Norm. CO_2 Low O_2	2.94	0.08	3.30	0.38

[1]Mean (n=22) calculated from the means for each treatment (n=20).

In terms of gas use efficiency the nitrogen, oxygen and carbon dioxide cylinders in the high-pressure manifolds only needed filling every 20, 31 and 70 days respectively. Thus a total of 35 nitrogen, 9 oxygen and 8 carbon dioxide cylinders were used to fumigate 80 chambers for the 140 day experiment. The fumigation system achieved its design objectives and the overall efficiency of the system and ease of use makes it suitable not only for this experiment but provides for further research opportunities using different plant species and gas fumigation regimes. The system can be used for relatively rapid primary screening of plant species for suitability for landfill revegetation and further research into the chronic effects of plants to different soil gas mixtures.

5.1 MATERIALS AND METHODS

5.3.1 Plant materials and treatment

One year old saplings of *Barringtonia racemosa* and *Harpephyllum caffrum* were supplied from the local nursery. The saplings of each species were carefully chosen so as to be of similar height and condition. They were supplied in 500ml plastic potting bags containing

standard potting soil (90% pine bark). In order to differentiate between old roots and new roots, the root balls, with the original potting soil, were lightly teased and placed into a nylon net bag (6mm square mesh). The trees were planted into the 20*l* fumigation chambers uniformly packed with a 1:1 sieved topsoil: washed river sand mixture. A river sand topsoil mixture was used so as to ensure even gas distribution during fumigation and easier removal of the soil medium from the roots at the end of the experiment. So as to ensure successful transplantation, the condition of the trees was monitored for a month before the chambers were closed and fumigation began. A Kelway 16-F soil moisture meter was used for weekly checks on soil moisture within each fumigation pot and moisture levels were kept constant by the addition of an appropriate amount of water.

There were twenty chambers per treatment with 10 replicate trees per species. The trees were fumigated for 140 days from the 8th of January 2001 to 27 June 2001 with the following treatments: "normal" soil O_2 (20%) and CO_2 (2%); high CO_2 (25%) and normal O_2 (20%); low O_2 (3%) and normal CO_2 (2%); high CO_2 (25%) and low O_2 (3%). Unfortunately the treatments could not be randomly positioned within the greenhouse as the distance of the chambers, within each treatment, from the distribution manifolds needed to be equal to ensure equal gas distribution. However, the greenhouse was relatively small and measurements of light intensity and air temperature showed no significant differences ($p>0.05$) between the areas in which the chambers for each treatment were positioned.

5.3.2 Measurement of above ground structure and development

The stem diameter of the trees, measured with digital callipers, was calculated from the mean of two diameter measurements taken perpendicular to each other at 5cm from the point of entry into the fumigation chamber for each stem. Stem height was measured from the point of stem entry into the fumigation chamber to the tip of the tallest apical shoot. The increase in stem diameter and height of each tree was determined by subtracting the original tree size from that of subsequent measurements. The number of leaves on each tree at the beginning and end of the experiment as well as any leaf loss during the experiment was also recorded. The overall condition of the plants was observed on a daily basis and any stress responses, such as epinastic curvature of the leaves or wilt were recorded. The final oven dry mass (105°C) of the above ground plant material was determined at the end of the experiment. Dry stem and leaf mass were determined separately so as to provide information about plant resource allocation. A sample of three leaves of similar age, which had developed during the experimental period, was taken from a similar position on 10 trees of each species within each treatment. The leaf area was measured using a CI 251 Leaf area meter (CID, Inc. NW Camas, Washington, U.S.A.), and dry mass was used to calculate leaf area mass ratios.

5.3.3 Physiological measurements

Stomatal conductance, A-Ci response curves (assimilation rate plotted against intercellular CO_2 concentration), and light response curves were measured using an open gas exchange system (LI-COR Portable Photosynthesis System -LI-6400, Li-Cor Inc., Lincoln, U.S.A.). Stomatal conductance was measured on a single fully mature leaf in the second whorl of the plant from each treatment for both species (n=10). Each set of measurements was completed within a morning and repeated approximately every 2 weeks during the experimental period. Measurements were taken only on sunny days and required 4 hours to

complete all 80 plants. Therefore, sampling had to be done in order to compensate for any possible changes in illumination due to movement of the sun or clouds. This was done by taking readings from only one plant per species in each treatment at one time. After all the treatments had been sampled in this manner, measurements were repeated in a similar way until all 80 plants had been sampled (Arthur, *et al* 1981). Other than the flow rate within the measuring chamber, the environmental conditions were not controlled and the stomatal conductance was measured as soon as ΔH_2O stabilised to less than 1%.

In order to determine the A-Ci response curves the chamber light (1500µmol m^{-2}, saturation point for both species), and leaf temperature (28°C) conditions were kept constant. The chamber CO_2 concentrations were varied from 100 µmol mol^{-1} to 2000 µmol mol^{-1} and the relative assimilation rates and intercellular CO_2 concentrations measured when the total coefficient of variation (sum of the coefficient of variation of ΔCO_2; ΔH_2O and Δflow) was less than 1%. For the Light Response Curves the chamber CO_2 level (384 µmol mol^{-1}); leaf temperature (27°C) and humidity (15 mmol mol^{-1}) were kept constant and light intensity was varied from 2000 µmol m^{-2} s^{-1} to 0 µmol m^{-2} s^{-1} and the rate of assimilation was measured when the total coefficient of variation was less than 1%. The A-Ci and Light Response Curves were measured on leaves of similar age (mature leaf within the second whorl) of 5 plants for each species within each treatment, before treatment, after 30 days, 90 days and after 140 days of treatment. Data collected for the individual plants at any set interval were fitted with a line [equation: $y = a(1-\exp(b-c*x))$] using regression analysis and the generated constants were accepted if the R^2 value was > than 0.9. The mean of the constants a, b and c for the individual species within the treatments were used for comparison between treatments using an analysis of variance and multiple range test (Sheffe, $p<0.05$).

5.3.4 Leaf nutrients

Leaf samples were sent to the KwaZulu-Natal Department of Agriculture Soil Fertility and Analytical Services for the following analyses: Total leaf content of Ca; Mg; K; Na; P; Zn; Cu; Mn. The procedures used were based on that described by Hunter (1974). Leaf samples were dry ashed at 450°C overnight. The samples were then cooled and wet with few drops of distilled water, and 2ml of concentrated HCl was added to each 1g sample. Samples were dried on a water bath and then 25ml of 1M HCL solution was added using a Fortuna Optifix dispenser and then filtered through a Whatman No. 41, 9cm filter paper.

The reagent used for Ca; Mg; K and Na determination was a strontium solution consisting of 76g $SrCl_2.6H_2O$ in 10l of de- ionized water. Using a diluter a 1ml aliquot of the filtrate was added to 24ml of the strontium solution, this was then used to determine element content with the following instrument settings of a Varian Spectra A 220FS atomic absorption spectrophotometer. Ca was determined at 422.7nm, current of 4mA and a slit width of 0.5nm; Mg at a wavelength of 285.2nm, current of 4mA and slit width of 0.5nm; K at a wavelength of 766.5nm, current of 5mA and slit width of 1nm; and Na at a wavelength of 589.0nm, current of 10mA and slit width of 0.5nm. For the determination of Zn, Cu and Mn the undiluted filtrate in 1M HCL was used with the following instrument settings: Zn, Cu and Mn were all determined at a current of 5mA and a slit width of 1nm with wavelengths of 213.9nm; 324.8nm and 279.5nm used respectively.

In order to determine leaf phosphate concentration a 2ml aliquot of the filtrate strontium solution was added to 8ml de-ionized water and 10ml of P colour reagent. This was allowed to stand for 30 minutes and read on a spectrophotometer at 680nm. The P colour

reagent was prepared by making a solution with 15g ammonium molybdate ($(NH_4)_6Mo_7O_{24}4H_2O$)) in 600ml distilled water. This was added to an acid antimony solution, made up of 2g antimony potassium tartrate ($C_4H_4KO_7Sb$. ½H2O) with 800ml distilled water and 300ml concentrated H_2SO_4, and made up to a volume of 2*l* with distilled water. From this stock solution the P colour reagent was made by diluting 150ml of the stock solution with 1l of a solution containing 1g gelatin and 1g ascorbic acid.

5.3.5 Root morphology

After the experimental treatment period a 10cm wide vertical section of the fumigation chamber wall, of each chamber, was removed for observing rooting pattern. The exposed profile was covered with clear plastic and the pattern of the exposed roots traced. The traced profiles were then used to determine the maximum root branching order for each tree so as to determine if any differences in rooting response was caused by the different soil gas treatments. The branching habit of roots has very important implications for the performance of a root system in unfavourable conditions. The development of lateral roots starts within the protective cylinder of the main root endodermis, thus a reduction in laterals can be indicative of stress within the root cortex (Scott Russell, 1977)

The vertical sections of the chambers were replaced and held in position with wide adhesive tape. Six of the 10 replicates / species / treatment were divided into 5cm horizontal intervals (Total 7 segments per pot). Each 5cm section was successively removed from the chamber top using an angle grinder, which allowed the slicing of the pot wall, soil and roots to be completed accurately and efficiently. The soil from each section was washed through a 2mm sieve so as to separate the roots. For the upper two 5cm

intervals which intersected with the original net bagged root ball, only the soil and roots on the outside of the bag were removed at this time. This ensured that the new root growth under experimental treatment conditions was collected separately. The roots collected from each of the 7 sections and from within the net bag were oven dried at 105°C and weighed separately. The root mass was expressed as a ratio of the soil volume within each profile section in order to compensate for the slightly conical shape of the pot. These data as well as the root mass per section were used to determine a root biomass depth profile, maximum rooting depth and total root biomass. In determining the root mass depth profile there was a concern that the slight conical shape of the fumigation chambers may bias the results, therefore the data was analysed using root mass and root mass expressed as a ratio of soil volume. No difference in the results between the two ways of measuring the root profile was found, the more meaningful root mass results are presented here. The root system for the other 4 replicates was kept intact and the soil was carefully washed from the roots. These roots which were then sampled for porosity and microscopy measurements. After porosity and microscopy measurements were completed the sampled root material was dried and weighed and added to the dry weight of the remaining root material for each of the 4 replicates, providing total root biomass data for each plant.

5.3.6 Porosity

Four replicate 5cm sections of stem sampled just above the fumigation chamber and fresh seminal root material were used for tissue porosity measurements. Porosity measurements were done using Archimedes' principle as described by Raskin (1983). The principle states that the buoyant force acting upon a body immersed in a fluid is equal to the weight of the fluid displaced by the body. Thus, by determining the mass of a body in air, then measuring the positive buoyancy mass of the body in water of known density (0.99707g

cm^{-3} at 25°C) before and after air space evacuation, the porosity of the body may be calculated using the following equations:

$$V_m = W_{air} - B_a \; / \; D_{water}$$

$$V_a = B_b - B_a \; / \; D_{water}$$

$$\text{Percentage Porosity} = V_a \; X \; 100 \; / \; V_m$$

V_m : Volume of material
V_a : Volume of air space
W_{air} : Weight in air
B_a : Buoyancy weight before infiltration
B_b : Buoyancy weight after infiltration
D_{water} : Density of water

The fresh mass of the stem and root material (n=4) was first determined in air and then the buoyancy mass of the material was determined in water (25°C). The gases within the material were displaced by vacuum infiltration (60Kpa) of 0.05% Triton X-100 surfactant solution, which has a density that can be considered equal to water for this experiment. The vacuum was applied and released 4 times to ensure complete infiltration of the root sections. The buoyancy mass of the infiltrated material was measured and the mass measurements in conjunction with water density (0.99707g cm^{-3} at 25°C) were used to calculate the percentage tissue porosity using the aforementioned equations.

5.3.7 Tissue anatomy

Fresh samples of stem tissue (3 replicates per species per treatment) were collected just above the chamber lid. Cross sections were taken with a sledge microtome and the woody tissue was viewed under the light microscope. Fresh root samples were taken from the seminal roots of the 4 replicate intact root systems for microscopy. Using hand-sections

and light microscopy the root sections were photographed and the root tissue structure was described.

5.2 RESULTS

5.4.1 Growth

There was no plant mortality in the fumigation experiment for either of the species and overall the trees appeared relatively healthy after 140 days. Both species across the treatments produced an average of only 3 new leaves during the experimental period with no particular treatment or species having a significantly ($p>0.05$) different amount of leaf loss. There was also no significant ($p>0.05$) difference between treatments for both species in terms of the total number of new leaves; total new leaf area; new leaf mass; or for the new leaf area / mass ratio. In terms of the final mass of plant material, after 140 days, *Barringtonia* showed no significant ($p>0.05$) difference between the treatments in total tree mass, total leaf mass, stem mass or original root mass within the net bag (Figure 5.12). However, the high CO_2 low O_2 and the high CO_2 normal O_2 treatments had significantly ($p<0.05$) lower new root mass relative to the control, suggesting elevated CO_2 was limiting *Barringtonia* root production (Figure 5.12). In *Harpephyllum* the high CO_2 low O_2 treatment resulted in a significantly ($p<0.05$) lower total plant mass compared to the control (Figure 5.13) and this was primarily due to a significantly ($p<0.05$) lower new root mass. Although the other two treatments also showed significantly ($p<0.05$) lower root mass relative to the control, they showed an equivalent mass increase in the stem resulting in no significant ($p>0.05$) difference in total plant mass (Figure 5.13). It appeared that elevated CO_2 or low O_2, alone or in combination caused a reduction in *Harpephyllum* root mass, however, the combination of elevated CO_2 and low O_2 was not accompanied by what appeared to be compensatory increase in stem mass.

The rate of stem height and diameter increase over the 140 days declined in all of the treatments including the control. This decline was expected, as newly potted plants will start off with a rapid growth that will stabilise with time. The decline was also attributed to the approach of winter and the shorter day lengths and lower sunlight intensity. In terms of the rate of height increase and the total height increase after 140 days there were no significant ($p>0.05$) differences between the treatments for either of the species (Figure 5.14 and 5.15).

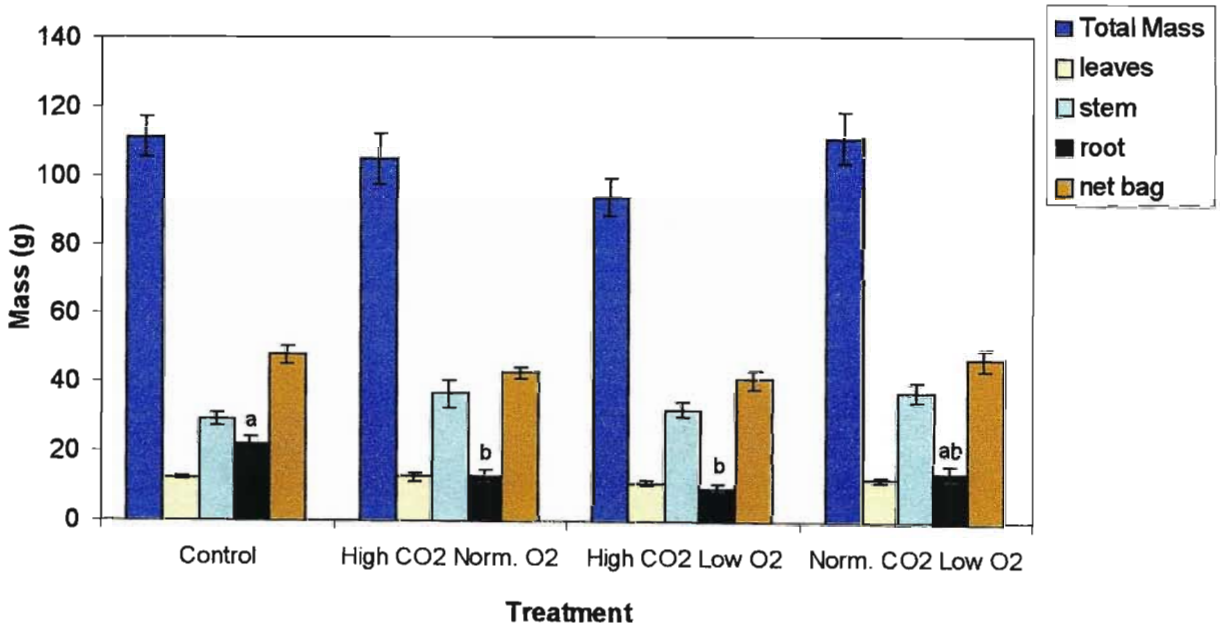


Figure 5.12: Tree total mass allocation for *Barringtonia* (n=10) after 140 days experimental treatment. Significant ($p<0.05$) differences between treatments for new root growth shown by a change in letter. There were no other significant differences between treatments.

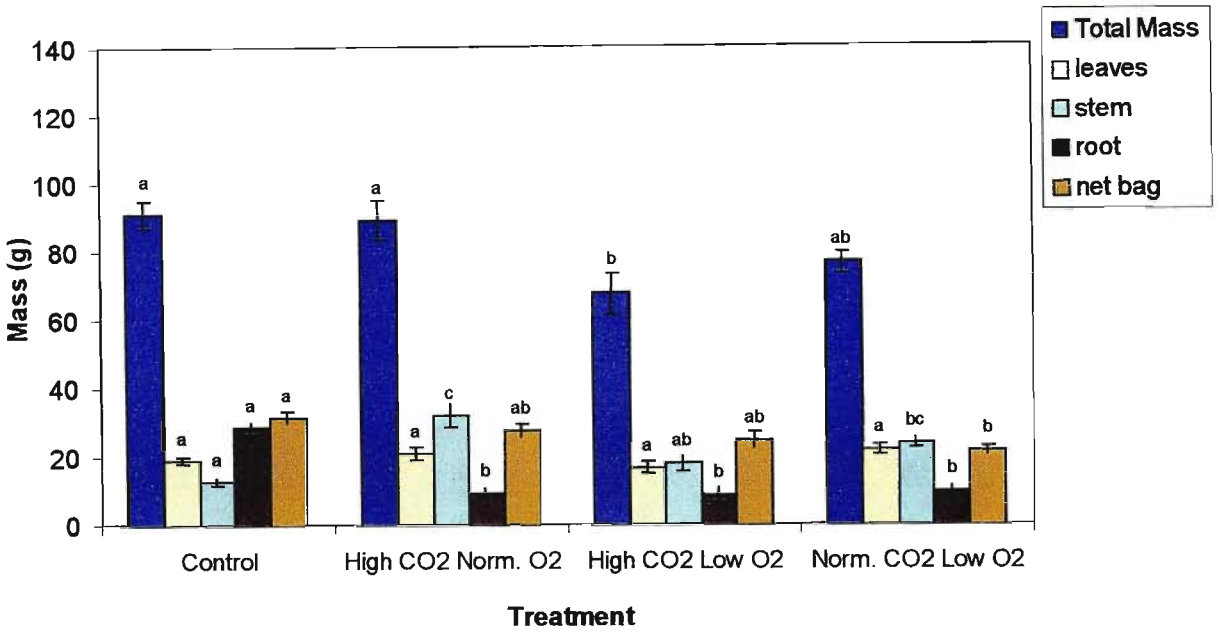


Figure 5.13: Tree total mass allocation for *Harpephyllum* (n=10) after 140 days experimental treatment. Significant ($p<0.05$) differences between treatments for each measured variable separately shown by a change in letter.

The results clearly showed that *Harpephyllum* had an initial higher rate of height increase relative to *Barringtonia* resulting in an overall higher total height increase. This initial high growth declined rapidly in the first 100 days, even under control conditions, until it was similar to *Barringtonia* growth rate. This response was attributed to a seasonal effect and was apparent in both species. In order to compare the species directly relative height increase was calculated by expressing the height increase of each tree as a percentage of the control mean for each species. *Harpephyllum* had a significantly lower relative height increase in comparison to *Barringtonia* in the elevated CO_2 low O_2 treatment and in the other treatments there was no significant ($p>0.05$) difference between the species (Figure 5.16). This suggested that the lack of enhancement of stem mass in the elevated CO_2 low O_2 treatment, as seen in the other treatments relative to the control, was partly caused by lower stem height increase.

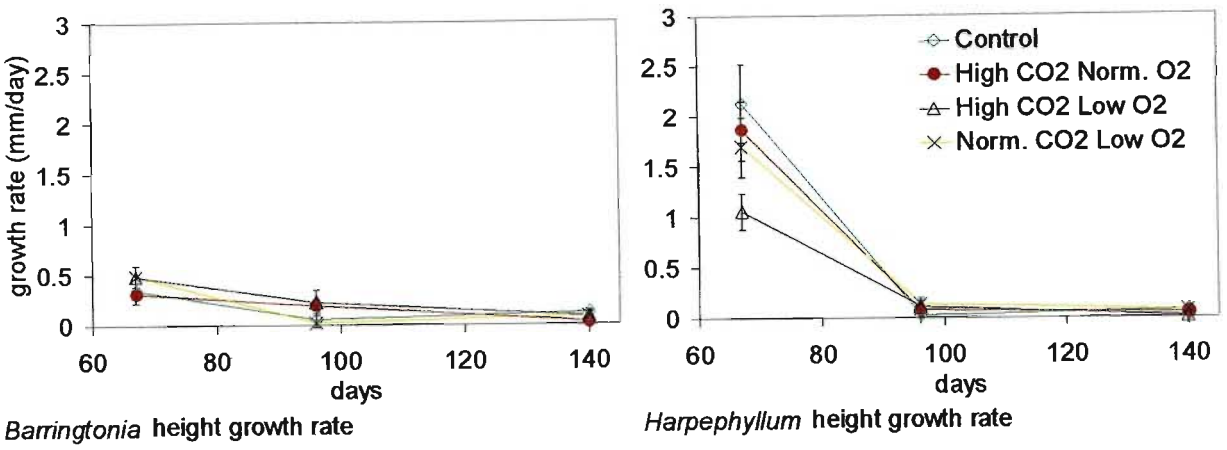


Figure 5.14: Rate of stem height extension for *Harpephyllum* and *Barringtonia*. No significant ($p>0.05$) differences between treatments at any point in time for either species.

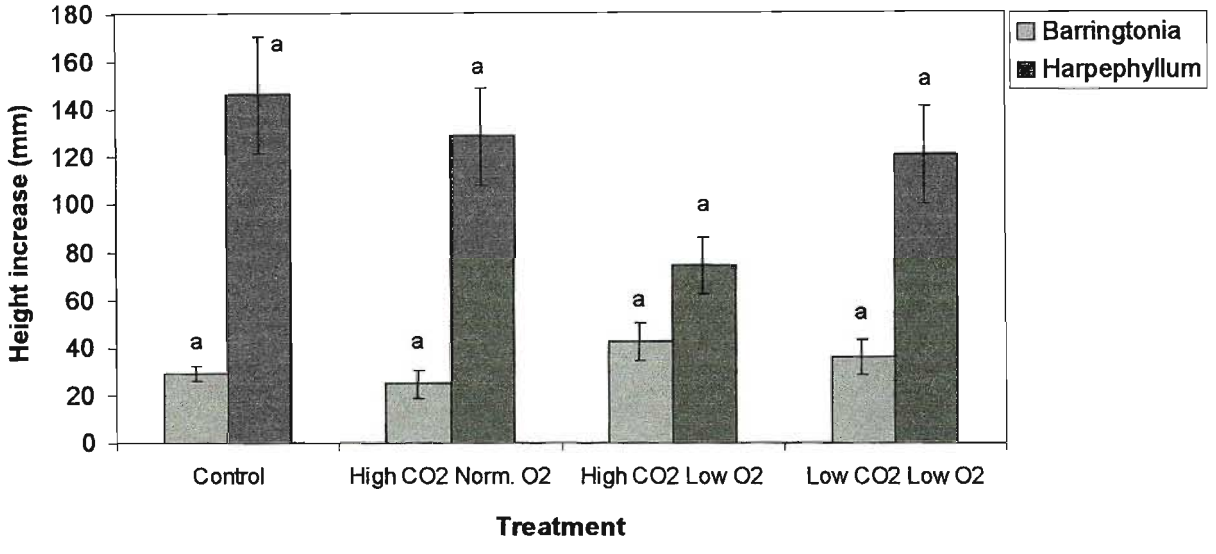


Figure 5.15: Total mean (±Std. Error) stem height increase after 140 days for *Barringtonia* and *Harpephyllum*. No significant ($p>0.05$) differences in height increase within species between treatments. Change in letter within species represents significant difference.

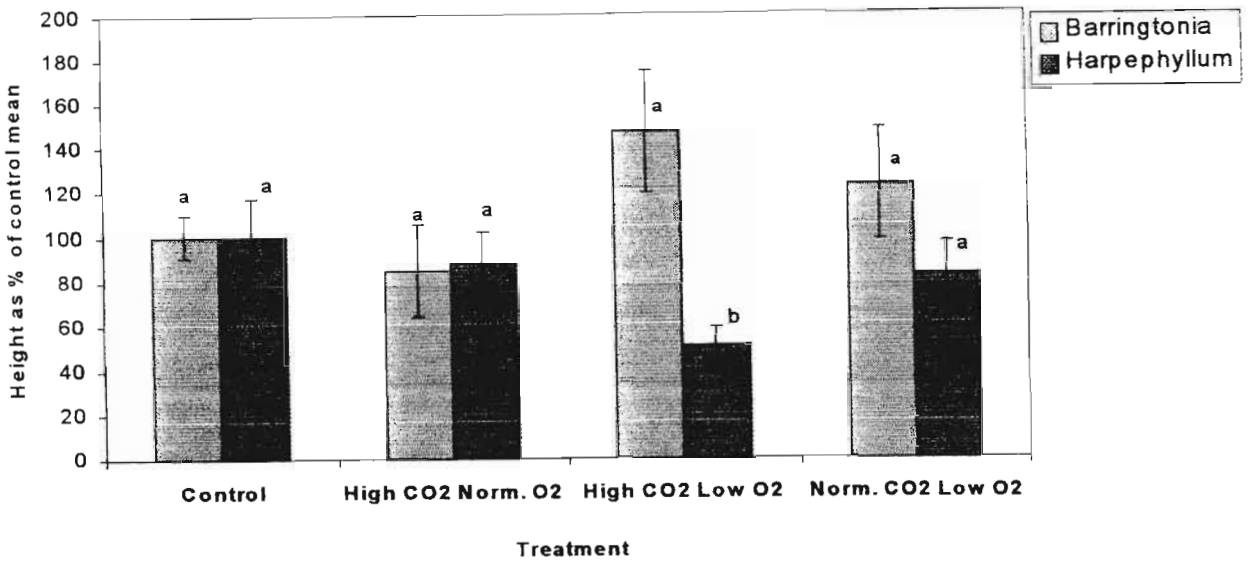


Figure 5.16: Total height increase expressed as a percentage of the control used to compare species within treatments. Significant ($p<0.05$) differences for each species within treatments shown by a change in letter.

In terms of the rate of stem diameter increase, *Barringtonia* in the normal CO_2 low O_2 treatment had an initial significantly ($p<0.05$) higher rate of increase measured at 70 days. This rate was not maintained and after 100 days the rate was not significantly ($p>0.05$) different from the control (Figure 5.17a). However, this initial high rate of stem diameter increase resulted in a significantly ($p<0.05$) higher total stem diameter increase relative to the control even after 140 days (Figure 5.17b). In the elevated CO_2 treatments the rate of stem diameter increase, although not initially as high as the normal CO_2 low O_2 treatment at 70 days, did not decline steeply and maintained a relatively high rate which became significantly ($p<0.05$) higher than the control when measured at 140 days. In fact the rate of stem diameter increase in the elevated CO_2 normal O_2 treatment was significantly ($p<0.05$) higher than the normal CO_2 low O_2 treatment. However, due to the initial low rate of increase in the elevated CO_2 treatments the actual total stem diameter increase over the 140 days was higher but not yet significantly ($p>0.05$) different from the control. The results suggested elevated CO_2 caused a slower, more sustained increase in the rate of stem diameter increase, even in the presence of low O_2, whilst low O_2 and normal CO_2 caused

an initial very high but non-sustained rate of stem diameter increase. Given a longer experimental period the rates of diameter increase suggest that the diameters of both the elevated CO_2 treatments would become greater than the normal CO_2 low O_2 treatment.

In the normal CO_2 low O_2 and the elevated CO_2 normal O_2 treatments *Harpephyllum* had an initial significantly ($p<0.05$) high rate of stem diameter increase relative to the control but this was not maintained (Figure 5.18a). However, it did result in a significantly ($p<0.05$) higher total stem diameter increase in these treatments relative to the control (Figure 5.18b). This suggested that the increase in *Harpephyllum* stem mass discussed earlier was primarily due to diameter increase. In the elevated CO_2 low O_2 treatment there was no initial high rate of stem diameter increase relative to the control (Figure 5.18a). This resulted in no significant ($p>0.05$) difference in total stem diameter increase relative to the control and explained the lack of increase in stem mass. Unlike the other treatments which showed an increase in stem mass equivalent to the reduced root mass, which resulted in no difference in total mass relative to the control, the elevated CO_2 low O_2 resulted in no enhancement of stem mass resulting in a significantly lower total mass (Figure 5.13). The results show that *Harpephyllum* in the high CO_2 low O_2 treatment had no initially high stem diameter increase rate or higher stem mass and had an apparent lower stem height increase, whilst the other treatments showed a definite stem growth response. This suggested a possible synergistic effect resulting from the combination of elevated CO_2 with low O_2 causing no enhanced stem growth that was a response measured in the other treatments. In comparing the stem diameter of the two species *Harpephyllum* had an overall greater stem diameter, however, in terms of relative stem diameter increase *Barringtonia* had a significantly ($p<0.05$) higher increase in the elevated CO_2 low O_2 and the normal CO_2 low O_2 treatments. Both species showed a similar stem growth response to

treatment conditions, however *Barringtonia's* response was slower and more sustained and was not inhibited by the combination of elevated CO_2 and low O_2.

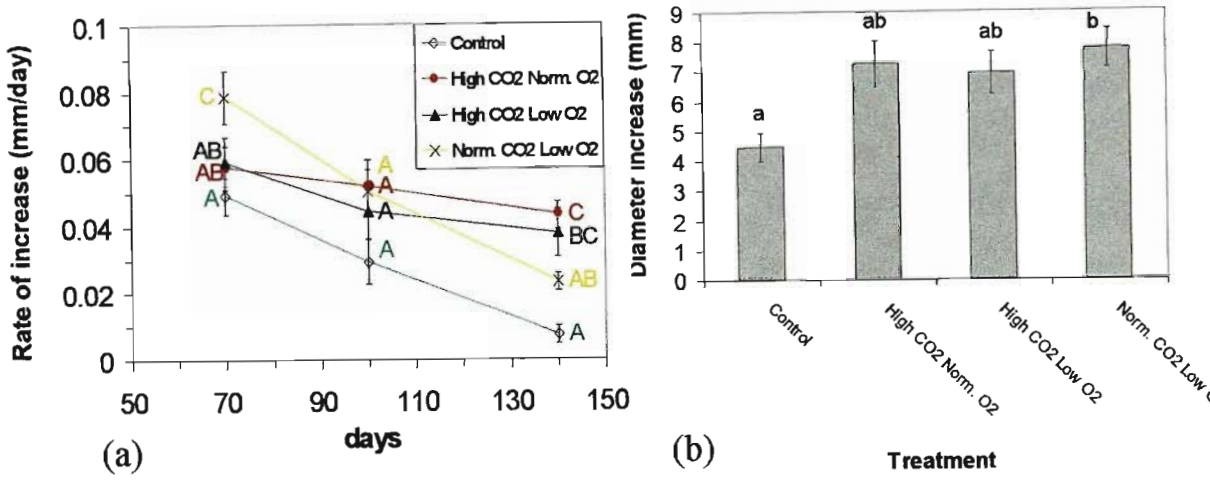


Figure 5.17: (a) Rate of *Barringtonia* stem diameter increase during experiment. Significant ($p<0.05$) differences between treatments at any individual point in time shown by a change in letter. (b) Total stem diameter increase after 140 days (i.e. $T_{140} - T_0$) in *Barringtonia*, significant ($p<0.05$) differences between treatments shown by a change in letter.

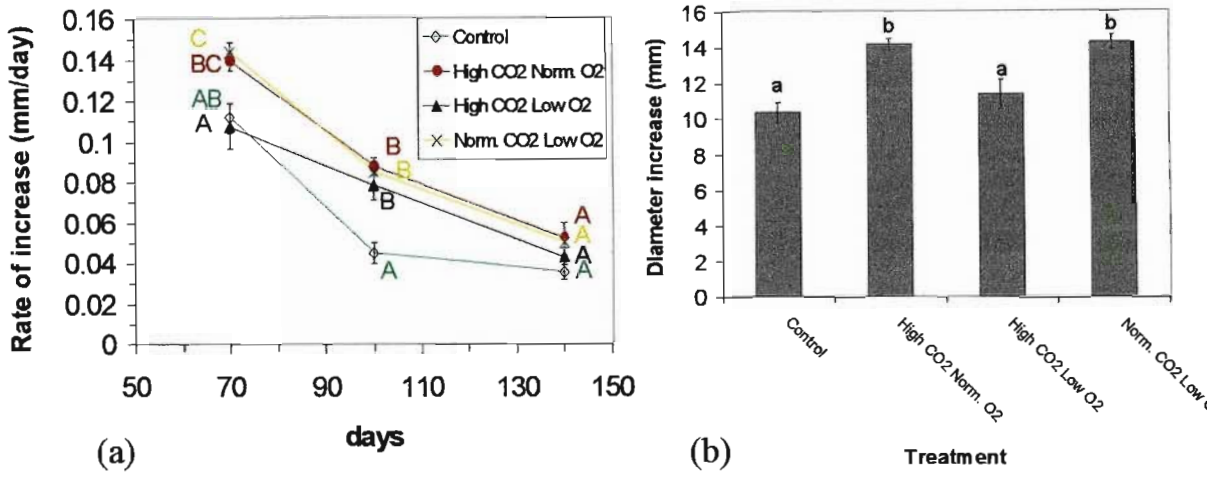


Figure 5.18: (a) Rate of *Harpephyllum* stem diameter increase during experiment. Significant ($p<0.05$) differences between treatments at any individual point in time shown by a change in letter. (b) Total stem diameter increase after 140 days (i.e. $T_{140} - T_0$) in *Harpephyllum*, significant ($p<0.05$) differences between treatments shown by a change in letter.

5.4.2 Physiology

In terms of the physiological measurements made significant responses of both species to the treatments were only observed after more than approximately 75 days. This indicated that the response of these species to the CO_2 and O_2 soil conditions could be most accurately called a chronic response.

Stomatal conductance, as for the rate of stem diameter increase and height growth, declined with time throughout the treatments for both species (Figures 5.19 & 5.22). From the first week of the experiment the stomatal conductance in the normal CO_2 low oxygen treatment was usually the lowest of the treatments and after 111 days it was consistently, significantly ($p<0.05$) lower than the control (Figure 5.19). In the Light and Aci response curves there were no differences between the treatments for the mean regression [$y = a(1-\exp(b-c*x))$] constants "a", "b" or "c" at any point during the experiment using a significance limit of $p<0.05$. However, using $p<0.1$, the mean "a" regression constant from the 140 day light response curves, in the normal CO_2 low O_2 treatment, was significantly lower than the other treatments (Figure 5.20). Although not significant ($p>0.1$) the Aci response curves also indicated that the normal CO_2 low O_2 treatment was having the most effect on *Barringtonia* physiology (Figure 5.21). These results suggest that low oxygen was influencing the rate of carbon assimilation in *Barringtonia*, however the presence of elevated CO_2 (i.e. the high CO_2 low O_2) appeared to prevent this effect.

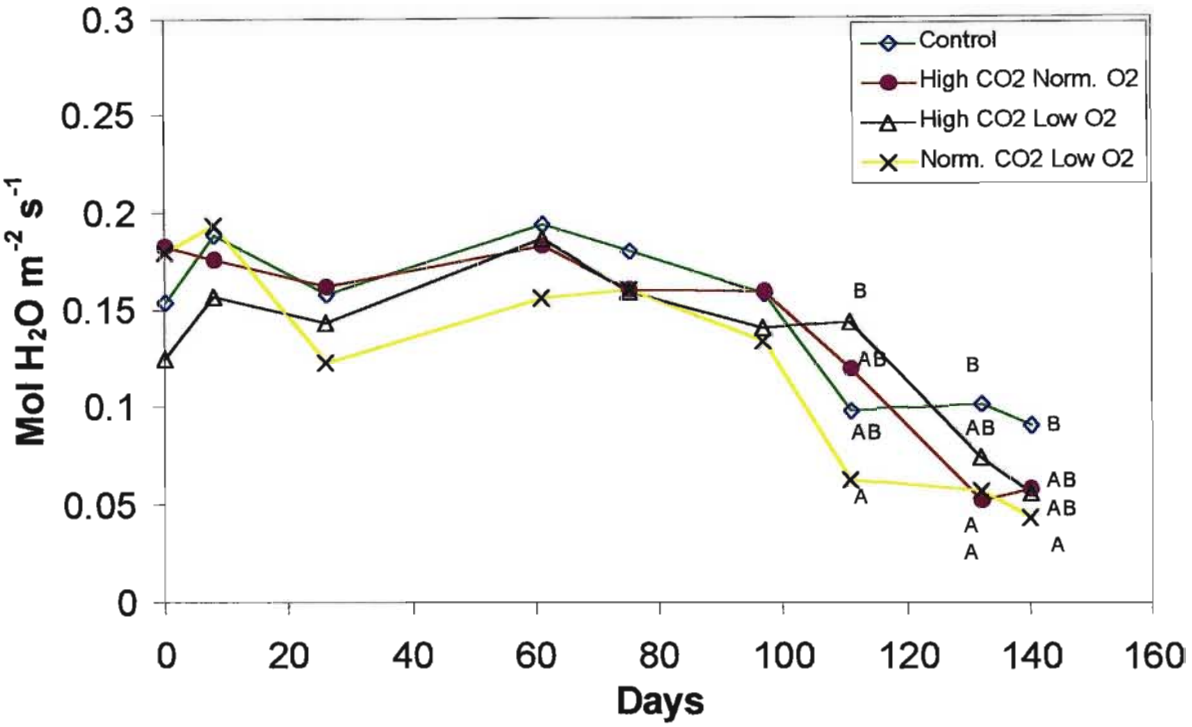


Figure 5.19: *Barringtonia* stomatal conductance, no significant ($p>0.05$) differences between treatments up to 111 days, significant ($p<0.05$) differences between treatments shown by a change in letters.

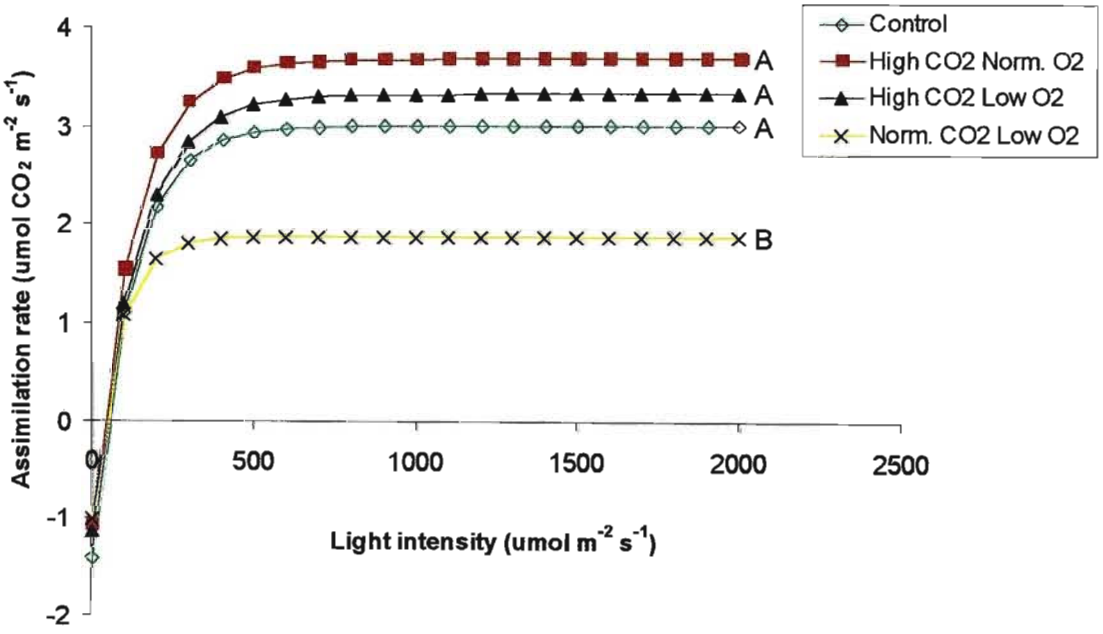


Figure 5.20: *Barringtonia* light response curves (140 day) for the different treatments. Significant ($p<0.1$) differences in regression constant "a" shown by a change in letter. There were no significant ($p>0.1$) differences in the regression constants "b" and "c".

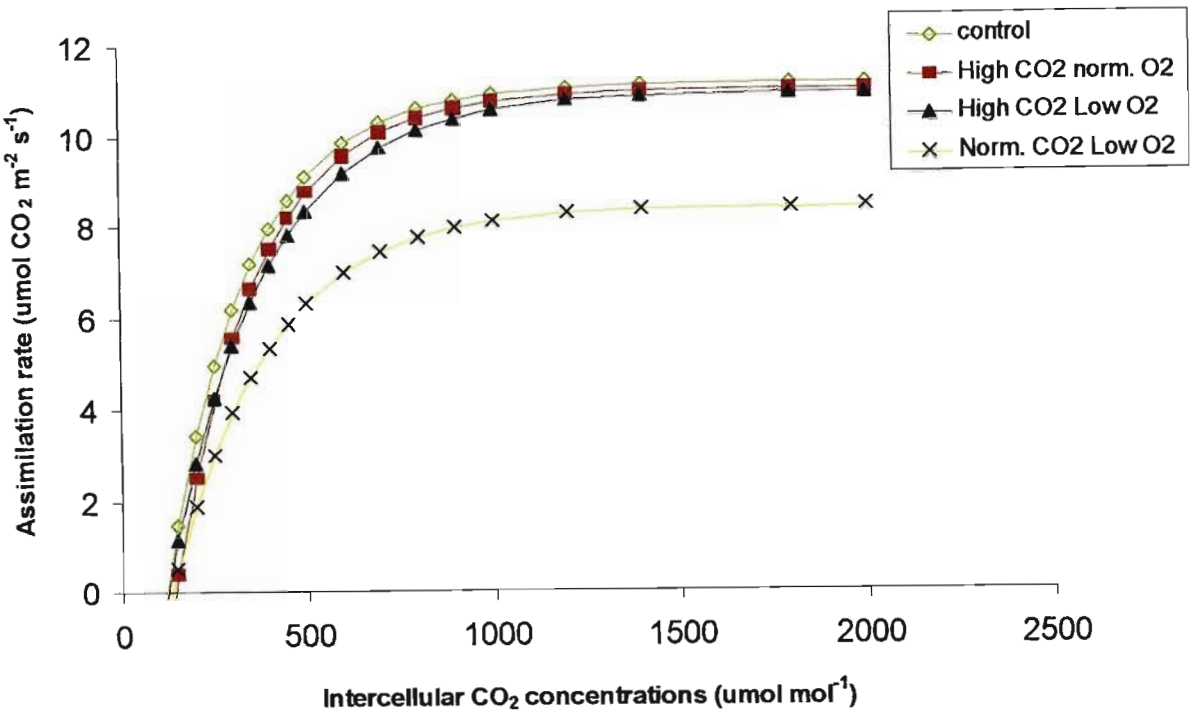


Figure 5.21: *Barringtonia* Aci response curves (140 day) for the different treatments. No significant ($p>0.1$) differences in regression constants.

Unlike *Barringtonia*, the stomatal conductance of *Harpephyllum* in the normal CO_2 low O_2 treatment showed an initial decline but the difference relative to the control became non-significant ($p>0.05$) after 111 days of fumigation (Figure 5.22). Also unlike *Barringtonia* the light response and Aci curves for the normal CO_2 low O_2 treatment were very similar to the control, with no significant ($p>0.1$) difference in the regression constant values at any point in time. However, after 75 days of fumigation the elevated CO_2 treatments showed significantly ($p<0.05$) lower stomatal conductance values relative to the control (Figure 5.22). There was only one exception at 111 days when there was no significant difference ($p>0.05$) in stomatal conductance between any of the treatments. This was attributed to relatively high data variability, due to patchy cloud cover, and was not considered an important break in the general trend. In fact stomatal conductance in the elevated CO_2 treatments dropped to 0.02 mol H_2O m^{-2} s^{-1} at 140 days and prevented the accurate

calculation of intercellular CO_2 concentrations and Aci response curves could no longer be generated, thus data for Aci response curves are not given. However up to this point there were no significant differences ($p>0.1$) in Aci regression constants between the treatments. The light response curves also showed no significant ($p>0.1$) differences, except for the 140 day measurements which showed a significantly ($p<0.1$) lower regression constant "a" in the elevated CO_2 treatments relative to the control (Figure 5.23). Interestingly the "a" constant in the low O_2 treatment without elevated CO_2 did not differ significantly ($p>0.1$) from the control. The results suggested that *Harpephyllum* stomatal conductance and the light response were affected by elevated CO_2 and there was no response to low O_2, whilst *Barringtonia* appeared to be most affected by the low O_2 conditions and elevated CO_2 appeared to alleviate this effect. *Harpephyllum* had significantly ($p<0.05$) lower relative "a" constant in the elevated CO_2 treatments whilst in the normal CO_2 low O_2 treatment *Barringtonia* had a significantly ($p<0.05$) lower relative "a" constant in comparison to *Harpephyllum*. The relative stomatal conductance results, although not significant ($p>0.05$) also suggested that elevated CO_2 was having a greater impact on *Harpephyllum* than *Barringtonia*, whilst low O_2 in the absence of elevated CO_2 was having an effect on *Barringtonia*.

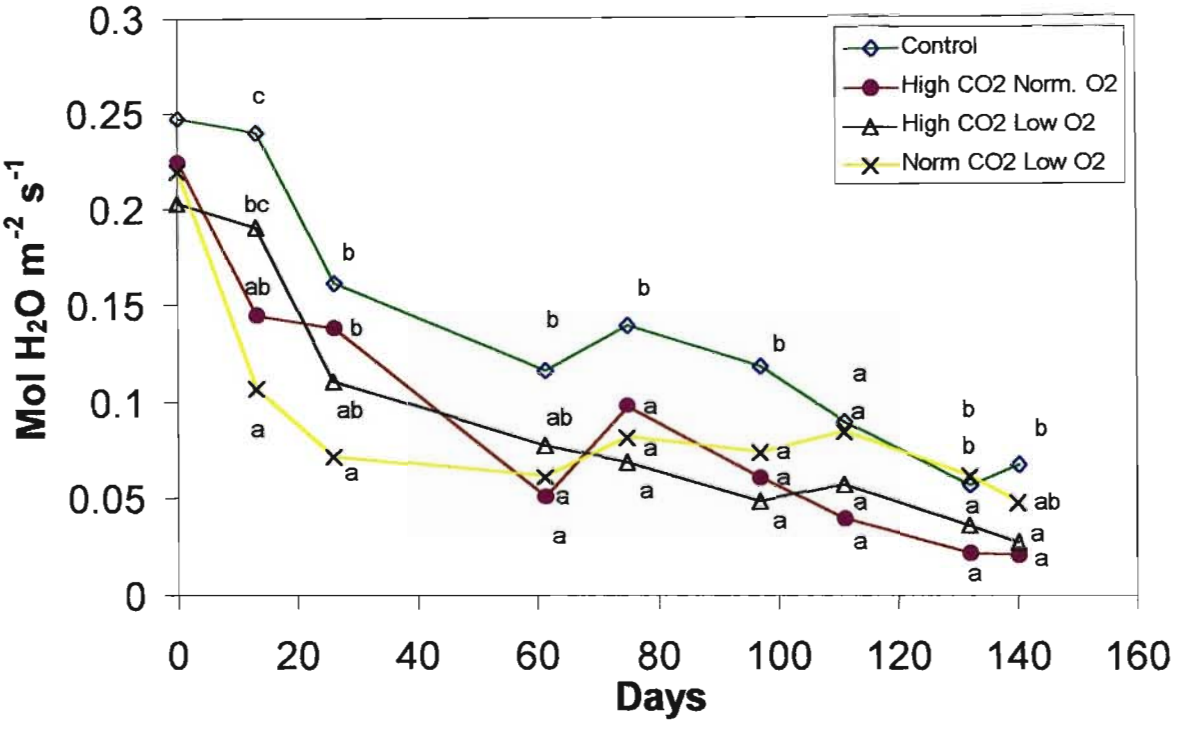


Figure 5.22: *Harpephyllum* stomatal conductance, significant ($p<0.05$) differences between treatments at any point in time shown by a change in letter.

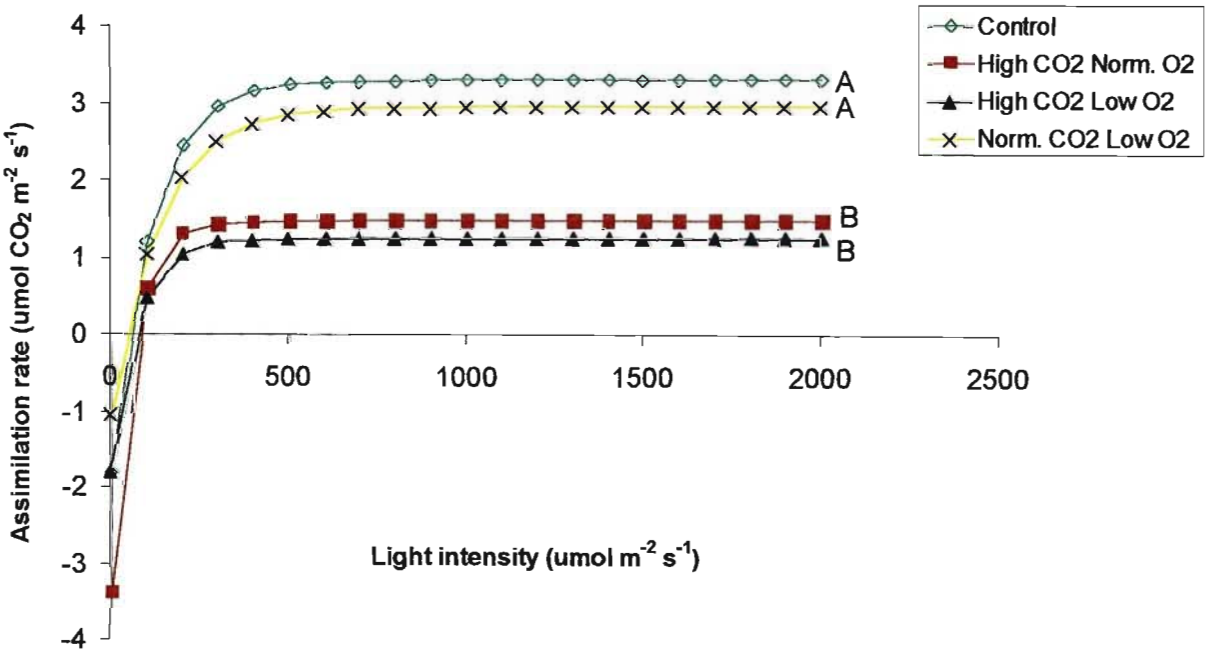


Figure 5.23: *Harpephyllum* light response curves (140 day) for the different treatments. Significant ($p>0.1$) differences in regression constant "a" shown by change in letter.

The leaf nutrient levels of both species were affected by the fumigation treatments, and are given in Tables 5.2 and 5.3. In the elevated CO_2 low O_2 treatment *Barringtonia* had significantly lower Mg levels and higher Na levels (Table 5.2), whilst *Harpephyllum* had significantly lower K levels, relative to the control (Table 5.3). In terms of relative nutrient content, there was no difference in Mg content between the two species however, *Barringtonia* had significantly higher K and Na levels in comparison to *Harpephyllum* for all of the treatments (Table 5.4).

In *Harpephyllum* there was a distinctive pattern of low leaf nutrient content associated with the elevated CO_2 normal O_2 treatment relative to the control and high leaf nutrient content associated with elevated CO_2 low O_2 relative to the elevated CO_2 normal O_2 treatment. This was especially noticeable for K, Mn, Cu, Mg and P (Table 5.3). The results suggested that elevated CO_2 was causing a lower leaf nutrient content and low O_2 was having an antagonistic impact on this effect. In *Barringtonia* there was little pattern in the results to indicate which gas was having the most marked effect. For example Cu levels were significantly lower than the control in the high CO_2 normal O_2 however there was no significant ($p>0.05$) difference between this treatment and the normal CO_2 low O_2 or high CO_2 low O_2 treatment which both did not vary significantly from the control (Table 5.2). Variations in the Mg and Na leaf content between the treatments provided the only insight into individual gas effects on *Barringtonia*. The variation in Mg levels between the treatments could suggest a reduction in levels in the leaves caused by elevated CO_2 and Na levels appeared to be higher under low O_2 conditions (Table 5.2).

Table 5.2: Mean nutrient concentrations (mg/Kg ± standard error) in *Barringtonia* leaves after 140 days of the different gas regimes. Significant ($p<0.05$) differences between treatments for each element shown by a change in letter.

Nutrient	Control	High CO_2 Norm. O_2	High CO_2 Low O_2	Norm. CO_2 Low O_2
Ca	13300 ±900 a	13200 ±600 a	11100 ±400 a	12200 ±800 a
Mg	7200 ±200 b	6300 ±500 ab	5500 ±300 a	6700 ±500 ab
K	6700 ±400 a	10100 ±1100 a	10000 ±900 a	7500 ±800 a
Na	600 ±40 a	1000 ±200 ab	1400 ±200 b	1300 ±200 b
P	1700 ±200 a	1200 ±100 a	1200 ±100 a	1200 ±200 a
Zn	72.3 ±6.8 a	63.7 ±7.5 a	65.3 ±8.7 a	60.4 ±5.6 a
Cu	11.8 ±1.4 b	6.0 ±1.0 a	8.5 ±1.5 ab	6.6 ±1.1 ab
Mn	248.3 ±16.2 a	276.3 ±30.6 a	225.1 ±19.4 a	235.1 ±21 a

Table 5.3: Mean nutrient concentrations (mg/Kg ± standard error) in *Harpephyllum* leaves after 140 days of the different gas regimes. Significant ($p<0.05$) differences between treatments for each element shown by a change in letter.

Nutrient	Control	High CO_2 Norm. O_2	High CO_2 Low O_2	Norm. CO_2 Low O
Ca	27000 ±1200 a	22800 ±1200 a	21200 ±1300 a	23400 ±1900 a
Mg	2000 ±100 ab	1600 ±200 a	1700 ±100 a	2400 ±200 b
K	9200 ±400 b	6600 ±500 a	7100 ±400 a	7500 ±400 ab
Na	400 ±80 a	300 ±40 a	500 ±40 a	500 ±50 a
P	900 ±60 a	500 ±30 b	900 ±60 a	800 ±60 a
Zn	37.2 ±4.1 a	24.9 ±4.4 a	40.6 ±6.8 a	27.0 ±4.2 a
Cu	6.0 ±0.3 ab	5.0 ±0.4 a	5.5 ±1.0 a	8.5 ±0.6 b
Mn	49.1 ±5.5 ab	31.3 ±1.9 a	46.7 ±4.4 ab	53.1 ±4.1 b

In terms of relative nutrient content compared between the species within the treatments there were few significant ($p<0.05$) differences. However, of these differences it was noted that the *Barringtonia* generally had higher relative leaf nutrient contents except in the case

of Cu and Mn (Table 5.4). In general the leaf nutrient results suggested that the effect of the treatments was less marked in *Barringtonia* as only 3 nutrients compared to 5 in *Harpephyllum* had any significant differences between the treatments and the overall impact of the gas conditions was relatively vague.

Table 5.4: Comparison between species of leaf element content expressed as a percentage of the mean of the control for each species.

Element	***Barringtonia***		***Harpephyllum***		
Ca	**Mean %**	**Std. Error**	**Mean %**	**Std. error**	***P value***
High CO_2 Norm. O_2	99.3	4.3	84.4	4.3	0.028 *
High CO_2 Low O_2	83.3	3.0	78.5	4.8	0.402
Norm. CO_2 Low O_2	91.8	5.9	86.6	6.9	0.582
Mg					
High CO_2 Norm. O_2	87.7	6.6	80.7	11.6	0.596
High CO_2 Low O_2	76.4	4.4	84.7	4.6	0.204
Norm. CO_2 Low O_2	93.7	6.9	116.7	9.3	0.67
K					
High CO_2 Norm. O_2	150.9	17.0	72.3	5.7	0.001 *
High CO_2 Low O_2	148.9	13.3	77.5	4.8	0 *
Norm. CO_2 Low O_2	112.3	12.2	81.6	4.9	0.026 *
Na					
High CO_2 Norm. O_2	165.7	28.8	84.4	10.5	0.023 *
High CO_2 Low O_2	240.7	31.9	112.5	9.3	0.001 *
Norm. CO_2 Low O_2	220.3	28.4	115.0	11.3	0.002 *
P					
High CO_2 Norm. O_2	70.8	7.0	58.9	3.4	0.163
High CO_2 Low O_2	68.5	4.6	108.0	6.9	0 *
Norm. CO_2 Low O_2	68.8	9.3	87.4	6.7	0.118
Zn					
High CO_2 Norm. O_2	88.1	10.4	66.9	11.8	0.196
High CO_2 Low O_2	90.3	12.0	109.1	18.3	0.401
Norm. CO_2 Low O_2	83.6	7.8	72.6	11.3	0.443
Cu					
High CO_2 Norm. O_2	50.8	8.8	83.3	6.3	0.01 *
High CO_2 Low O_2	72.0	12.7	91.7	17.3	0.372
Norm. CO_2 Low O_2	55.6	9.3	141.7	10.0	0 *
Mn					
High CO_2 Norm. O_2	111.3	12.3	63.6	3.9	0.003 *
High CO_2 Low O_2	90.7	7.8	95.1	8.9	0.71
Norm. CO_2 Low O_2	94.7	8.4	108.1	8.3	0.274

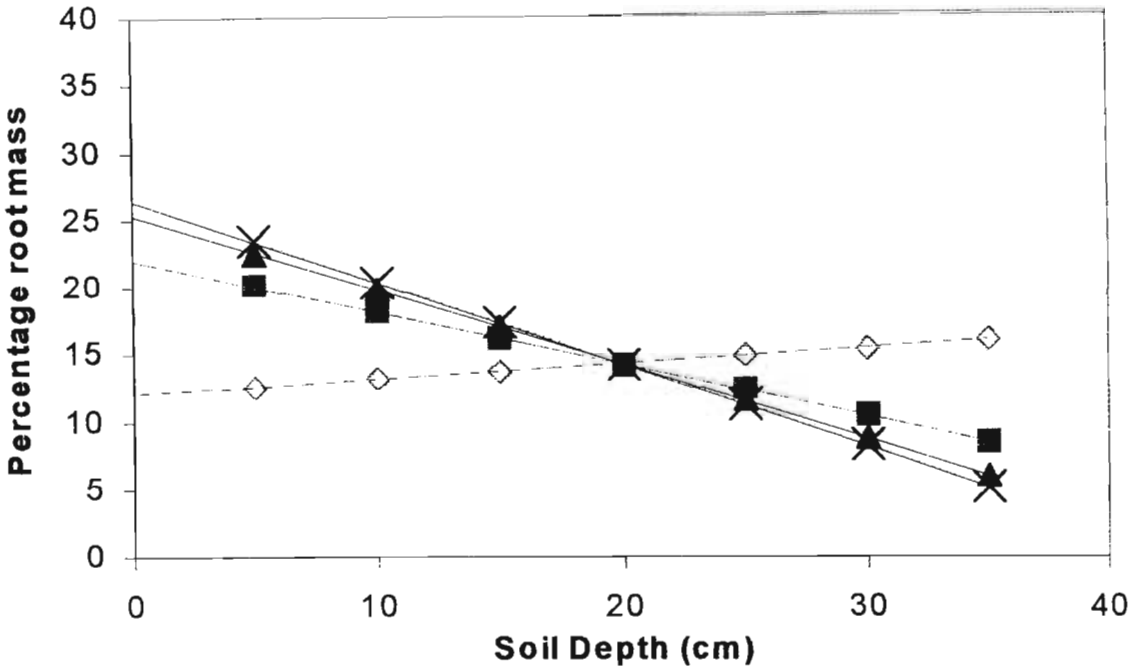
* significant difference between species (ANOVA $p<0.05$)

5.4.3 Root morphology

The mass of new roots within each of the 7 depth intervals was expressed as a percentage of the total new root mass. Using linear regression, the y intercepts and gradients for the root mass depth profiles of 6 trees / species / treatment were determined. The mean gradient and mean y intercept for each species for each treatment was calculated. A comparison of the mean gradients and mean y intercepts between treatments, within species, was made using analysis of variance. The regression lines generated from the mean gradient and y intercept for each treatment are shown for *Barringtonia* in Figure 5.24 and *Harpephyllum* in Figure 5.25. The depth profile for *Barringtonia* roots showed a significant ($p<0.05$) reverse in gradient relative to the control for the low oxygen treatments. Whilst *Harpephyllum* showed a significant ($p<0.05$) reverse in gradient for all the treatments relative to the control, indicating that the proportion of root mass reduced with depth instead of increasing.

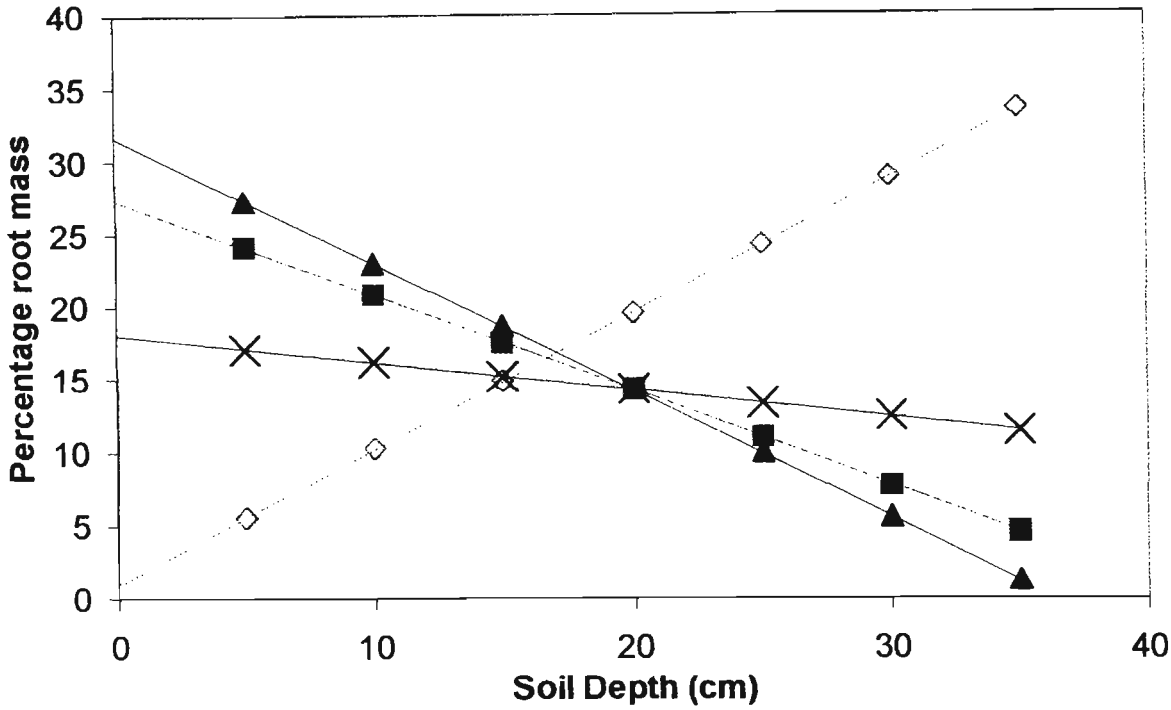
The y intercept values for *Barringtonia* indicated that the low oxygen treatments had significantly ($p<0.05$) higher proportions of root mass near the soil surface relative to the control, whilst the high CO_2 normal O_2 treatment was higher but not significantly ($p>0.05$) different from the control (Figure 5.24). In *Harpephyllum* the y intercept values indicated a significantly higher proportion of root mass near the surface for all the treatments relative to the control (Figure 5.25). The elevated CO_2 treatments had a greater proportion of roots mass near the surface relative to the normal CO_2 low O_2 treatment. However, only the elevated CO_2 low O_2 was significantly ($p<0.05$) different (Figure 5.25).

In summary, low oxygen conditions resulted in an overall greater proportion of roots near the soil surface for both species. However, *Harpephyllum*, unlike *Barringtonia*, had an even greater shift in the proportion of roots near the soil surface under elevated CO_2 conditions, even in the presence of normal oxygen levels. Although, the treatments had a significant effect on the root biomass depth profile of both species there was no apparent effect on the root branching habit. There were no significant ($p>0.05$) differences found between the mean ($n=10$) maximum root branching orders measured between the treatment or the species (Figure 5.26). The roots seen within the traced profiles showed an average of three levels of branching, however, it was not possible to establish if those roots seen were already of a higher branch order before they became visible in the exposed profile. Therefore the technique possibly lacked the ability to detect subtle changes in root branching that may have been caused by the treatments.



Treatment	Gradient	Y intercept
◇ Control	A	A
■ High CO2 Norm. O2	AB	AB
▲ High CO2 Low O2	B	B
× Norm. CO2 Low O2	B	B

Figure 5.24: *Barringtonia* root mass depth profile shown by regression lines generated from the mean gradient and y intercept for each treatment. Legend shows significant differences between treatments in gradient or y intercept of regression lines by a change in letters (Sheffe multiple range test).



Treatment	Gradient	Y intercept
◇ Control	A	A
■ High CO2 Norm. O2	B	BC
▲ High CO2 Low O2	B	C
× Norm. CO2 Low O2	B	B

Figure 5.25: *Harpephyllum* root mass depth profile shown by regression lines generated from the mean gradient and y intercept for each treatment. Legend shows significant differences between treatments in gradient or y intercept of regression lines by a change in letters (Sheffe multiple range test).

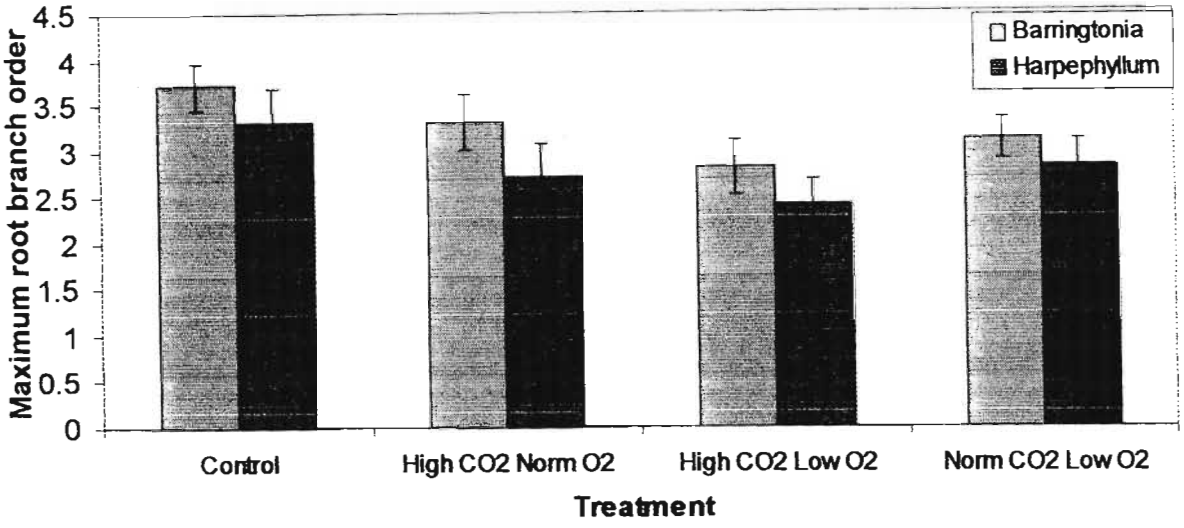


Figure 5.26: Mean (n=10) maximum root branch order for each treatment for both species. Error bar shows standard error of mean. No significant ($p>0.05$) differences between species or treatments.

5.4.4 Root and stem structure

The mean porosity of *Barringtonia* roots (range 8.9-13%) and stem (range 9.2-11.7%) was considerably, and significantly ($p<0.05$), higher across all treatments compared to *Harpephyllum* roots (range 0.1-2.3%) and stems (range 1.1-1.9%). There was no significant ($p>0.05$) difference between stem and root tissue porosity for *Barringtonia*, suggesting a possible high level of continuous interconnected intercellular air spaces in this species (Figure 5.27). The tissue porosity also appeared to be an inherent characteristic, as there was no significant ($p>0.05$) difference between any of the treatments (Figure 5.27). For *Harpephyllum* there was also no significant ($p>0.05$) difference in tissue porosity between the experimental treatments however, unlike the other treatments, in the control the roots had very low porosity and were significantly ($p<0.001$) lower than the stem (Figure 5.28).

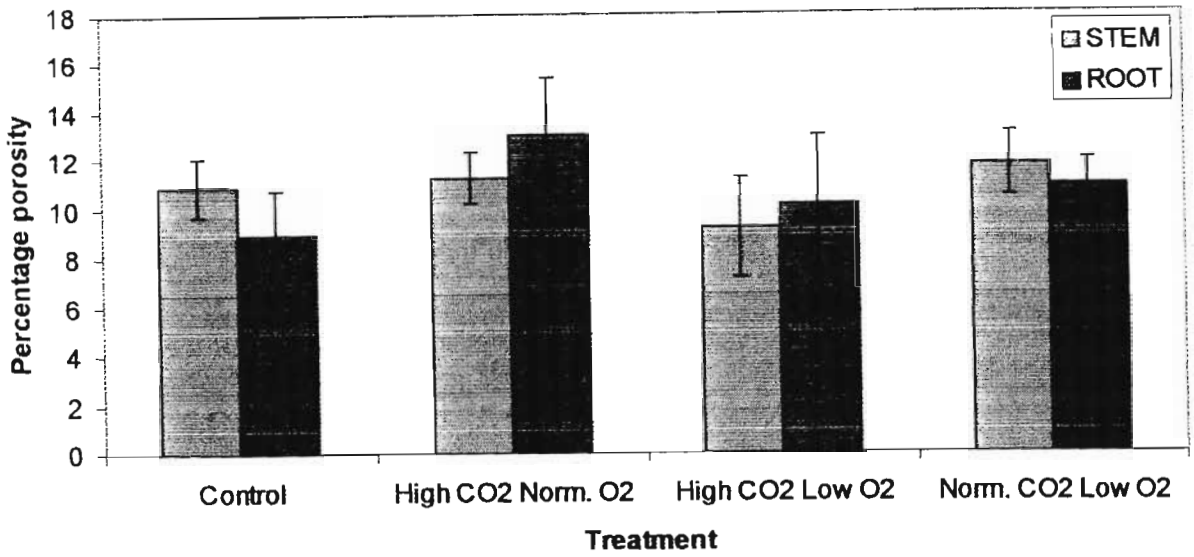


Figure 5.27: Mean with standard error of stem and root porosity for *Barringtonia*. No significant ($p>0.05$) differences between treatments or between stem and root within treatments.

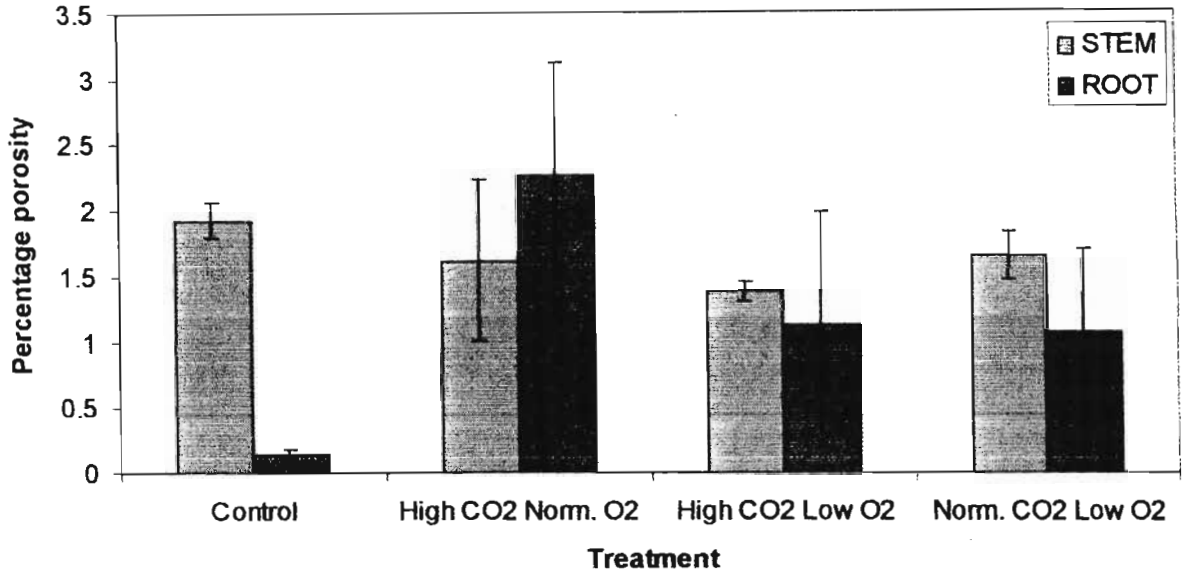


Figure 5.28: Mean with standard error of stem and root porosity for *Harpephyllum*. No significant ($p>0.05$) differences between the treatments or between root and stem within treatments, except for the control which had significant difference between stem and root ($p<0.001$).

These data for *Harpephyllum* suggests a possible increase in root porosity in the presence of gas treatments, however, the relatively large standard error of the mean root porosity

data, which explains the lack of significant difference between the control and the treatments, makes the data difficult to interpret. The results could suggest that the increase in root porosity was highly variable within the seminal roots indicating root cell die back rather than the formation of continuous interconnected intercellular air spaces.

The microscopical study of seminal root and stem material showed a marked difference in tissue structure between the two species. The key differences in the root tissue were the degree of secondary thickening and configuration of cortical cells. In *Barringtonia* the cortical cells were loosely packed in well ordered radial rows with each cell having four near neighbours to give a cubic cell packing arrangement and the appearance of successive concentric rings of cells within the cortex (Figure 5.29). The resultant intercellular spaces were shaped like a concave quadrangulus. The absence of single or several adjacent radial rows of cortical cells was apparent in all of the *Barringtonia* root tissue sampled, providing evidence of inherent lysigenous aerenchyma formation. No secondary thickening was apparent in any of the root sections observed for *Barringtonia* (Figure 5.29). There was no evidence of root anatomical differences between the treatments (Figure 5.31).

The *Harpephyllum* root tissue showed evidence of extensive secondary thickening and the formation of secondary xylem and phloem. The cortex and epidermis had been replaced by cork cambium that together with its derivatives, the phellogen and phellum, comprised the periderm. The cell structure of the root tissue appeared denser than that of *Barringtonia*, with very little intercellular air space and no evidence of aerenchyma formation (Figure 5.30). There was also no evidence of root anatomical differences between the treatments (Figure 5.32). There was no microscopical evidence to explain the high variability in root porosity in the root tissue exposed to the treatments.

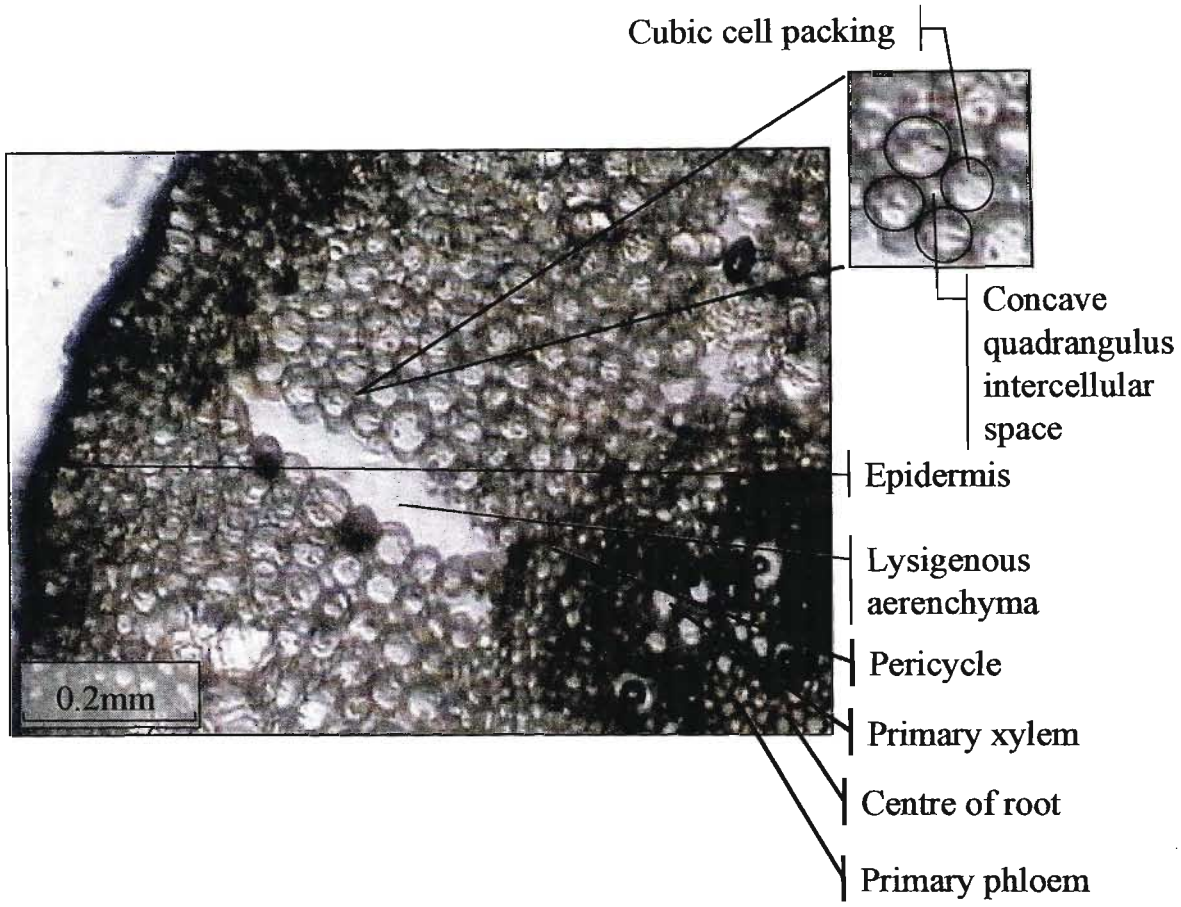


Figure 5.29: Cross section of *Barringtonia* seminal root showing cortical cell packing and the presence of aerenchyma

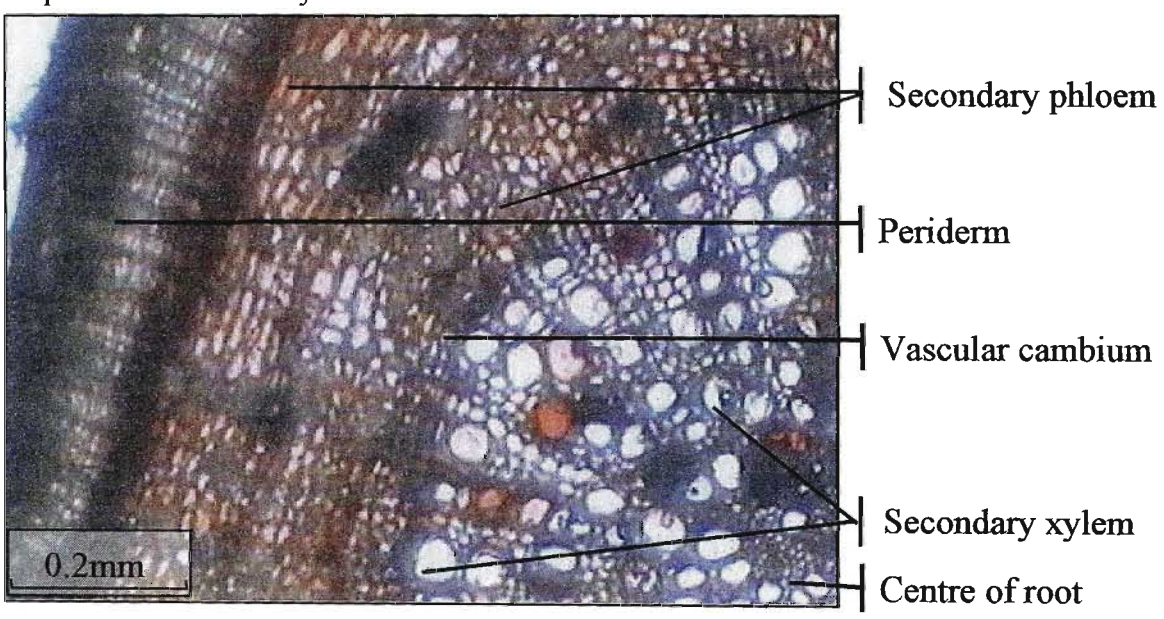
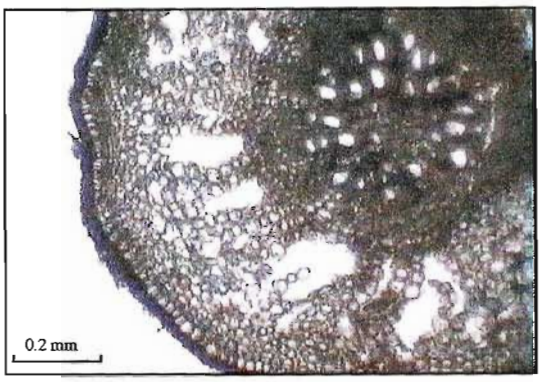
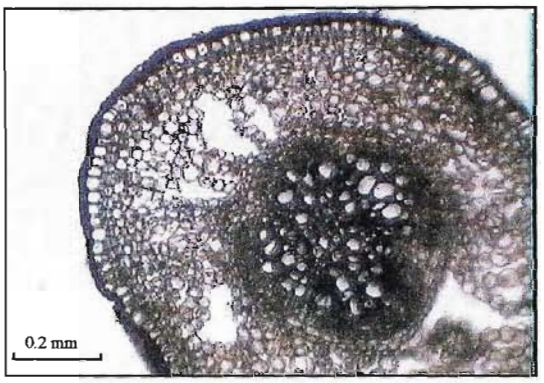


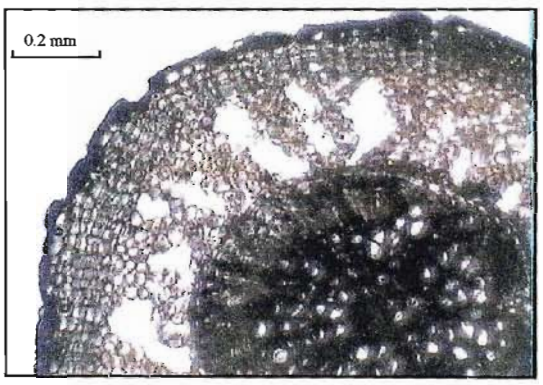
Figure 5.30: Cross section of *Harpephyllum* seminal root showing extensive secondary thickening and total loss of cortical cells and epidermis.



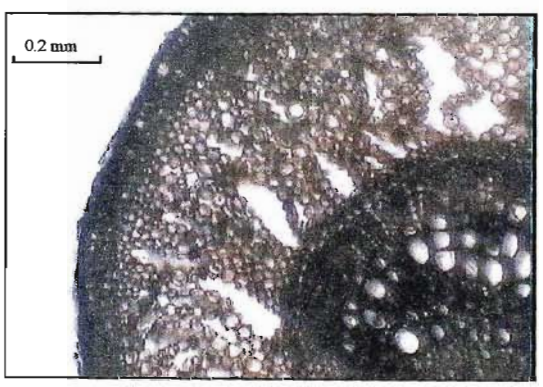
Control:
Barringtonia seminal
root cross section



High CO_2 Normal O_2:
Barringtonia seminal root cross section

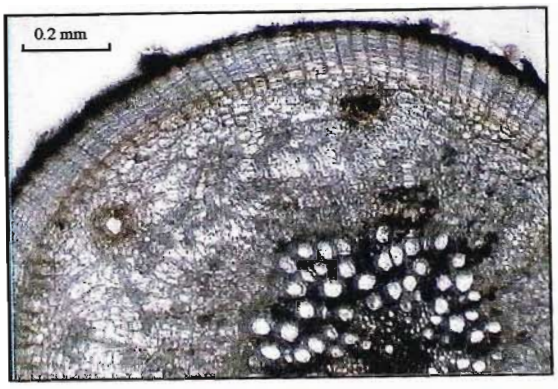


High CO_2 Low O_2:
Barringtonia seminal root cross section

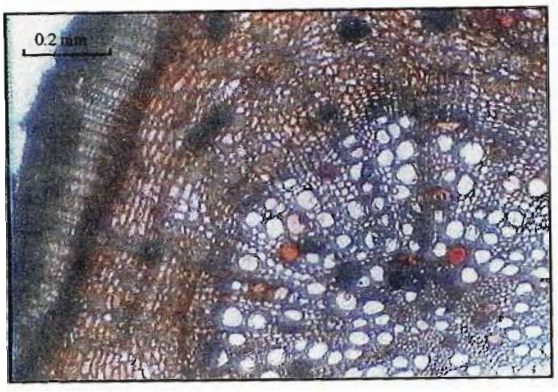


Normal CO_2 Low O_2:
Barringtonia seminal root cross section

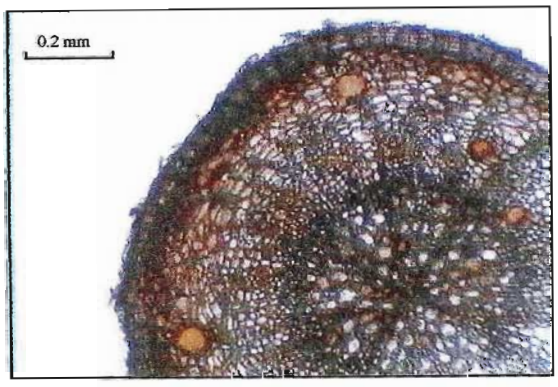
Figure 5.31: Light microscopy cross sections of representative examples of *Barringtonia* seminal roots from the different treatments, showing no treatment effect on root anatomy



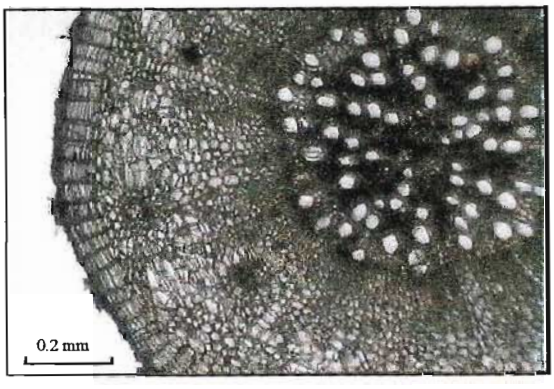
Control:
Harpephyllum seminal
root cross section



High CO_2 Normal O_2:
Harpephyllum seminal root cross section



High CO_2 Low O_2:
Harpephyllum seminal root cross section



Normal CO_2 Low O_2:
Harpephyllum seminal root cross section

Figure 5.32: Light microscopy cross sections of representative examples of *Harpephyllum* seminal roots from the different treatments, showing no treatment effect on root anatomy

In terms of the wood anatomy of the two species, there was a distinct difference in the level of wood fibres and overall tissue density. The *Barringtonia* stems had distinctly larger fibre cells and a more open wood structure when compared with the stem cross sections of *Harpephyllum.* (Figure 5.33 and 5.34). However, for both species there was no evidence of any differences in wood anatomy due to the treatments as seen in Figure 5.35.

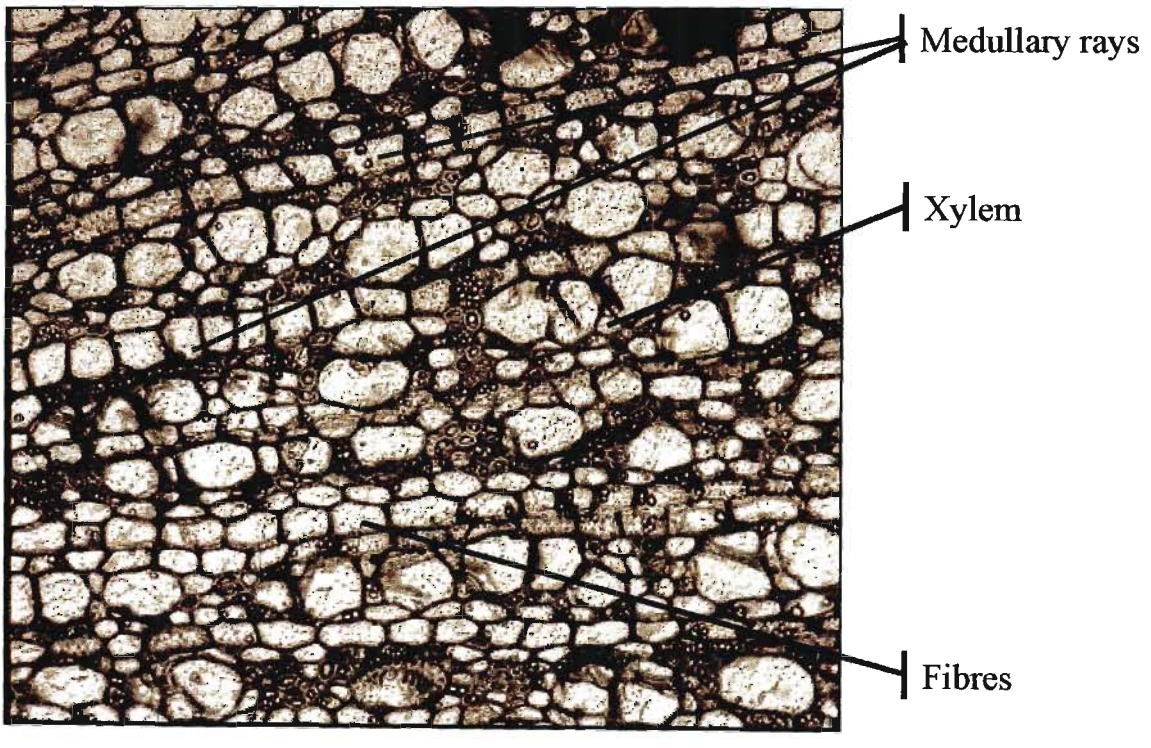


Figure 5.33: Cross section of *Barringtonia* stem, showing wood tissue structure (x25)

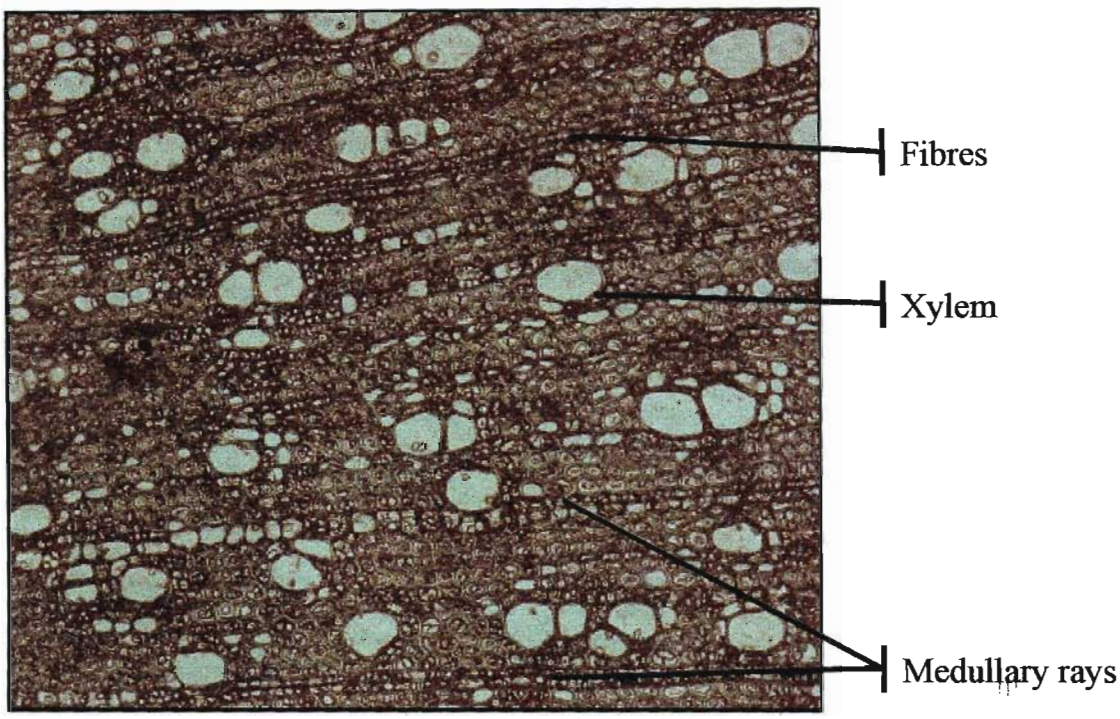
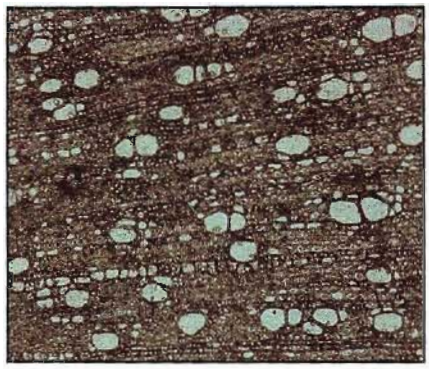
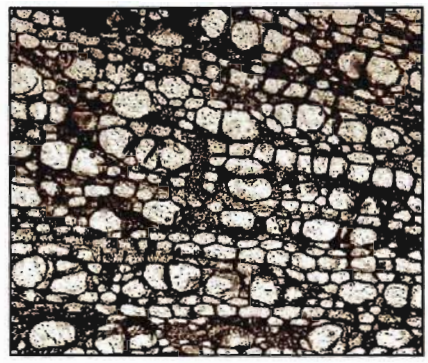


Figure 5.34: Cross section of *Harpephyllum* stem, showing wood tissue structure (x25)

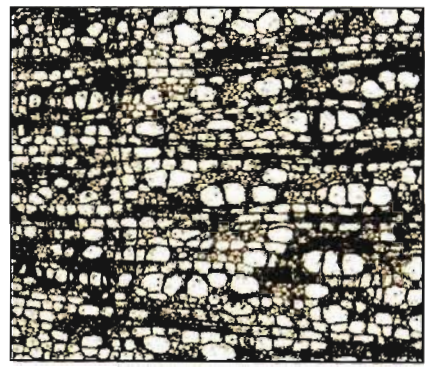


Control: *Harpephyllum*, stem cross section X 10



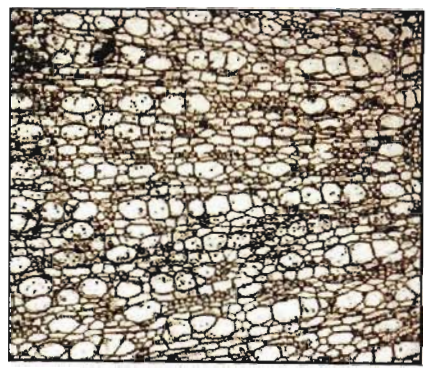
Control: *Barringtonia*, stem cross section X 10

High CO_2 Normal O_2: *Harpephyllym*, stem cross section X 10

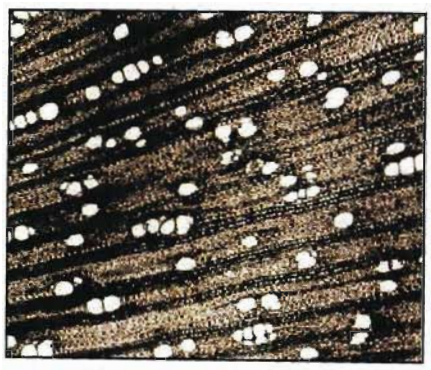


High CO_2 Normal O_2: *Barringtonia*, stem cross section X 10

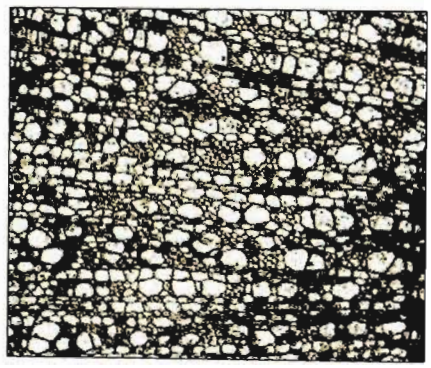
High CO_2 Low O_2: *Harpephyllym*, stem cross section X 10



High CO_2 Low O_2: *Barringtonia*, stem cross section X 10



Normal CO_2 Low O_2: *Harpephyllym*, stem cross section X 10



Normal CO_2 Low O_2: *Barringtonia*, stem cross section X 10

Figure 5.35: Cross sections of *Harpephyllum* and *Barringtonia* stem tissue showing no change in wood anatomy with treatment.

5.5 DISCUSSION

There were no mortalities or serious stress symptoms, such as epinasty or increased leaf loss, during the 140-day experiment, suggesting that elevated CO_2 (25%) and low O_2 (4%) concentrations used in this experiment do not cause an acute stress response. In terms of the physiological measurements made the response of both species to the treatments was only observed after approximately 75 days. This highlighted the importance of relatively long term investigations into the impact of landfill gas on plant species. It was also important that the plants were not killed by the treatments such that a detailed assessment of the plants functional response to the experimental conditions could be measured.

Since the early work of Leone *et al* (1977) it has been well documented that landfill gas pollution of the soil has a negative impact on plants. In accordance with previous observations the simulated landfill gas (i.e. elevated CO_2 low O_2 treatment) used in this experiment influenced the growth of both species, especially root growth. Similar simulated landfill gas experiments by Chan *et al* (1991) and Marchiol *et al* (2000) showed a reduction in plant root growth that was attributed to landfill gas induced stress. The observed reduction in root growth was not isolated to simulation experiments. The trees planted on the Bisasar Road landfill were exposed to high landfill gas concentrations and showed reduced rooting density (Chapter 4). Reduced root growth and has also been observed for trees planted at Edgeboro Landfill, New Jersey, where a significant negative correlation between high CO_2, low O_2 and total root length was reported (Gilman *et al* 1981).

The assessment of the relative effects of the separate components of the simulated landfill gas (i.e. normal CO_2 low O_2 and high CO_2 normal O_2 treatments) on root growth showed that high soil CO_2 (25%) despite good soil O_2 (20%) limited root growth for both species. Similarly Huang *et al* (1997) also found elevated soil CO_2 even with normal soil O_2 levels reduced root growth in both flooding sensitive and non-sensitive plants. Highlighting the distinct role that soil CO_2 can potentially have on plant growth. Elevated soil CO_2 appears to inhibit root respiration and modify carbon allocation (Conlin & van den Driessche, 2000; Nobel & Palta, 1989). This may be due to a decrease in cytosolic pH caused by soil CO_2 entering the cells by a hydration reaction (Nobel, 1990). Carbon dioxide levels in the root atmosphere ranging from 0.7% to 6.5% have been reported to cause inhibition of root respiration and growth in a number of different plant species by a number of researchers since as early as 1957 (Conlin & van den Driessche, 2000; Nobel, 1990; Nobel & Palta, 1989; Qi *et al*1994; Radin & Loomis, 1969; Stolwijk & Thimann, 1957). Therefore, the level of soil CO_2 (25%) used in this study was likely to have had an inhibitory effect on root respiration, thus explaining reduced root mass. Considering, a soil CO_2 level of 25% is not uncommon in landfill cover soils, reduced root respiration and growth is likely to be key factors influencing plant performance.

The relative effects of low soil O_2 (i.e. normal CO_2 low O_2) on root growth of *Barringtonia* was minimal, suggesting that reduced root mass seen in the simulated landfill gas treatment (high CO_2 low O_2) was primarily due to CO_2 (Figure 5.12). However, for *Harpephyllum* the root mass was reduced by low soil O_2 even in the presence of normal soil CO_2 levels (Figure 5.13). This is not unusual, as inhibition of root growth in response to soil oxygen deficiency has been reported for many vascular plant species (Kludze *et al* 1994). This reduced root growth is primarily due to inhibition of respiration and lack of an

electron acceptor, thus a shortage of ATP (Nobel & Palta, 1989). This can disturb the functional relationship between organs such as the roots and shoots (Drew, 1997; Vartapetian & Jackson, 1997). Generally soil O_2 levels below 10% restrict root growth and below 5% root growth ceases (Kozlowski, 1991). Considering the low oxygen treatments had mean O_2 levels between 3-5% the reduced root mass of *Harpephyllum* could be expected. It is apparent that the low soil O_2 conditions, like high soil CO_2, can have an inhibitory effect on root respiration. However, the fact that *Barringtonia* root mass was not influenced by the low soil O_2 conditions suggests that this species had mechanisms to manage the low soil O_2 conditions. These possible mechanisms will be considered when the root morphology and anatomy results are discussed later.

It is not uncommon for woody plants to experience a change in the rate of stem diameter growth in soils that become poorly aerated (Kozlowski, 1986). This is probably related to a shift in carbohydrate partitioning in response to root stress, causing changes in the growth rates of phloem parenchyma (i.e. increase in bark) and / or the number and size of xylem cells in the stem (Kozlowski, 1997). The reason or benefits of this response are unclear, and the response varies between species from temporarily or sustained accelerated growth rates to reduced growth (Kozlowski, 1986). This may provide an explanation for the variations in stem diameter growth, seen in this experiment, suggesting that the root stress created by the treatments was causing changes in the carbohydrate partitioning of the plants. *Harpephyllum* clearly showed a temporary increase in stem diameter growth in the high CO_2 normal O_2 and normal CO_2 low O_2 treatments (Figure 5.18). Whilst *Barringtonia* showed a temporary increase in the normal CO_2 low O_2 treatment and a more gradual but sustained higher rate of stem diameter growth in the treatments with high CO_2 (Figure 5.17). However, *Harpephyllum* showed little change in the rate of stem diameter growth in

the high CO_2 low O_2 treatment (Figure 5.18). Considering this treatment was also causing root stress, as seen by the reduced root mass (Figure 5.13), one would expect a shift in carbohydrate partitioning and a resultant change in stem diameter growth rate as seen in the other treatments. It was apparent that the combination of high CO_2 and low O_2 (simulated landfill gas) may be inhibiting a change in carbohydrate partitioning that was apparently induced by root stress in the other treatments.

When roots are depleted of O_2 it is important that the shoots respond metabolically to the root conditions and curtail their demand for root derived resources (Vartapetian & Jackson, 1997). The physiological measurements indicated that there was reduced metabolic activity of *Barringtonia* shoots in response to the normal CO_2 low O_2 treatment. The stomatal conductance of *Barringtonia* in the normal CO_2 low O_2 treatment was usually the lowest of the treatments from the first week of the experiment and after 111 days it was consistently and significantly ($p<0.05$) lower than the control. Under low soil O_2 conditions stomatal closure is possibly more than a passive response to poor water absorption by an energy deficient root system (Sojka & Stolzy, 1980). Jackson, (1994) and Smit *et al* (1990) suggested that the hypoxic status of roots is transmitted by an unknown signal in the transpiration stream resulting in a metabolic response in the leaves. The closure of the stomata and resultant low stomatal conductance under low soil oxygen conditions causes stomatal limitation of the photosynthetic system (Kludze *et al* 1994, Pezeshki *et al* 1996). This is not an uncommon phenomena, as stomata have an integral role in the regulation and control of photosynthesis (Jones, 1998). The depression of carbon assimilation and photosynthate utilization is an important response for maintaining the functional relationship between the roots and shoots and the 'management' of low O_2 soil conditions (Vartapetian & Jackson, 1997). The light and Aci response measurements clearly showed

lower carbon assimilation rates of *Barringtonia* in the normal CO_2 low O_2 treatment after 140 days. The apparent down regulation of leaf carbon assimilation would curtail shoot demands on root activity allowing for a functional equilibrium within the plant to be maintained and continued root function and growth. The reduced demand from the shoots for resources from the root would allow for root growth to be maintained, as illustrated by the lack of significant difference in the root mass between the control and the normal CO_2 low O_2 treatment (Figure 5.12).

Unlike *Barringtonia, Harpephyllum* in the normal CO_2 low O_2 treatment showed no evidence of stomatal limitation and reduced carbon assimilation in the shoots (Figure 5.22 and 5.23 respectively), however, root growth was significantly reduced. There was no evidence suggesting that shoot demand on the roots was alleviated which could have resulted in an imbalance in the functional equilibrium of the plant. If there were no means to alleviate the oxygen stress on the roots, the continued demand for resources by the shoots would result in reduced root growth, as seen by the reduced root mass (Figure 5.13). It is proposed with a longer experimental period (>140 days) a further deterioration of root growth and function would be observed and reduced stomatal conductance and carbon assimilation rates would inevitably occur due to photosynthetic system failure and not controlled down regulation.

Arthur *et al* (1981) found that the stomatal conductance of a tree species (*Acer saccharum*) with a known sensitivity to landfill soil conditions was significantly reduced by simulated landfill gas (3% O_2; 40% CO_2; 50% CH_4; 7% N_2). A field investigation by Gilman *et al* (1989) also showed that the elevated CO_2 and low O_2 conditions in landfill cover soils were associated with reduced stomatal conductance and lower plant growth in *Acer*

saccharum. Similarly in this study, *Harpephyllum caffrum*, a landfill sensitive species, showed a reduced stomatal conductance when the roots were fumigated with simulated landfill gas (i.e. high CO_2 low O_2). However, the results of this study suggested that the primary cause of reduced stomatal conductance of *Harpephyllum* was probably the high soil CO_2 and not low O_2. This was concluded because, unlike the normal CO_2 low O_2 treatment, the high soil CO_2 with or without low O_2 resulted in a significant reduction in stomatal conductance after only 75 days of fumigation and a reduced maximum carbon assimilation rate in the light response curve after 140 days. However, root growth was also significantly reduced by the treatments with elevated CO_2. The reduced root growth, indicated that the lower stomatal conductance and carbon assimilation rates were probably less likely to be a controlled down regulation of shoot demand on roots, as seen for *Barringtonia* in the normal CO_2 low O_2 treatment, and more likely a symptom of photosynthetic system failure. Considering that stomatal closure is usually one of the first responses to root stress (Liang *et al* 1995), it is possible that the physiological response of *Harpephyllum* to the elevated CO_2 treatment was due to CO_2 damage to root cells. In fact, the reduction in stomatal conductance became so severe that there was insufficient leaf – atmosphere gas exchange towards the end of the experiment for further measurements to be conducted. It was apparent that for *Harpephyllum*, high soil CO_2 possibly had a more rapid and marked effect than low soil O_2 on root function, resulting in a relatively more rapid limitation of carbon assimilation by shoots.

Further evidence that that high soil CO_2 had a more marked effect on *Harpephyllum* root function than low soil O_2 was provided by the leaf nutrient analysis. The results suggested elevated CO_2 and not low O_2 was causing lower leaf nutrient content, especially for K, Mn, Cu, Mg and P (Table 5.3). This indicated that high soil CO_2 was resulting in limited

nutrient uptake and / or transport to the shoots. This is not an uncommon phenomenon and Chang & Loomis (1945) showed reduced root uptake of nutrients due to elevated CO_2 in the root zone over 50 years ago. It was also noted by Ruark *et al* 1982 who attributed the lower nutrient uptake of roots to carbon dioxide toxicity, which decreased root permeability. In fact low O_2 appeared to have an antagonistic impact on the lower leaf nutrient content in *Harpephyllum* caused by elevated CO_2 (Table 5.3).

Although the elevated CO_2 treatments (high CO_2 low O_2 and high CO_2 normal O_2) also resulted in reduced root mass of *Barringtonia* the response of the species was different to *Harpephyllum*. Unlike *Harpephyllum* the high soil CO_2 conditions showed no clear impact on nutrient uptake. Also unlike, *Harpephyllum* the stomatal conductance and maximum assimilation rates in the light and Aci response curves did not vary significantly from the control after 140 days of the elevated CO_2 treatment. Similarly, Arthur *et al* (1981) found that stomatal conductance of a tree species (*Acer rubrum*) with a known 'tolerance' to landfill conditions was not affected by simulated landfill gas (3% O_2; 40% CO_2; 50% CH_4; 7% N_2). However, in this study it was also apparent that the elevated CO_2 was ameliorating the depression of stomatal conductance and carbon assimilation that was caused by the low soil oxygen conditions. The amelioration by elevated soil CO_2 of photosynthetic system depression caused by low soil O_2 has been observed, especially in species that are flooding tolerant (Huang *et al* 1997). However, the mechanism is not clear but several possibilities have been proposed such as changes in leaf ribulose–1,2-bisphosphate carboxylase-oxygenase or transport of root CO_2 through aerenchyma to shoots or the counter effects of CO_2 on ethylene inhibition (Huang *et al* 1997). These are discussed further below.

It was proposed by Arteca and Poovaiah, (1982) that Ribulose –1,2-bisphosphate could make use of CO_2 translocated from the roots to suppress photorespiration. This would reduce CO_2 production by respiration and result in higher apparent carbon assimilation as measured by leaf – atmosphere exchange in light and Aci response curves. The movement of CO_2 or other gases such as methane from the root to the shoot through aerenchyma has been demonstrated recently (Jackson & Armstrong, 1999; Le Mer & Roger 2001), thus the theory is not unreasonable. Arteca and Poovaiah, (1982) also showed that root zone application of CO_2 enhanced phosphoenolpyruvate carboxylase activity in the roots of some species, which can facilitate the fixing of root zone CO_2 into malate. This was confirmed by Gao and Lips, (1997) and they further showed that the malate produced was important for respiratory energy function and resulted in increased NO_3^- uptake. The increased NO_3^- was found to stimulate the transport of carbon assimilates to the shoot. Thus, it is possible that elevated root zone CO_2 was ameliorating the effects of low O_2 on root respiration allowing for a functional relationship with the shoots to be maintained.

The effects of ethylene inhibition by CO_2 is also a possible explanation for apparent ameliorative effects of elevated CO_2 on the impact of low O_2 seen in *Barringtonia*. Ethylene production by roots and soil micro-organisms is a common response to low levels of soil O_2, however high concentrations of ethylene may reach leaves via intercellular spaces affecting leaf physiology (Jackson *et al* 1987). Carbon dioxide has been shown to have an inhibitory effect on the influence of ethylene on plant metabolism (de Wild *et al* 2002; Radin & Loomis, 1969), thus it is also possible that elevated CO_2 reached the leaves via aerenchyma and prevented the impact of ethylene. It is not possible in this experiment to identify which mechanisms, if any, allowed *Barringtonia* to use CO_2 to escape the impact of low O_2 on shoot physiology. However, it is clear that aerenchyma as well as

enhanced enzyme activity within the plant could be of distinctive advantage for survival and growth in elevated CO_2 low O_2 soil environments such as those found in landfill cover soils.

A greater understanding of the mechanisms of root survival and growth in landfill soils was provided by the assessment of root morphology in this experiment. Both species showed a significant reversal in rooting depth gradient and a higher proportion of roots near the soil surface in the simulated landfill gas treatment (high CO_2 low O_2) relative to the control. However, it is important to note that the closed chamber design did not allow for atmospheric dilution of the gas treatment near the soil surface therefore, unlike in landfill cover soils in the field, shallower rooting would not allow avoidance of the high CO_2 and low O_2 conditions. There was also unlikely to be a soil moisture gradient within the chamber as loss of water through evaporation from the soil surface would also be minimal due to the closed chamber design. This suggests that in this fumigation experiment the shallower rooting was not simply a response to an environmental gradient within the chamber soil, but a distinct plant response to the soil atmosphere conditions. Interestingly, the results suggested that in *Harpephyllum* shallower rooting was primarily driven by the high soil CO_2, whilst in *Barringtonia* low soil O_2 appeared to be the key factor. Elevated soil CO_2 and not low O_2 has been reported as the main cause of shallower root growth for plants with normal sensitivities to CO_2 and O_2 in landfill cover soils (Chan *et al* 1991; Gilman *et al* 1981). This indicated that *Barringtonia* was unlike most other species and avoidance of high soil CO_2 did not appear to be of primary importance in its rooting response in this experiment. This concurred with the physiological results that showed little effect of high soil CO_2 on *Barringtonia* physiology.

Leone *et al* (1983) screened 19 tree species for landfill tolerance on a New Jersey landfill, the results indicated that the relatively 'tolerant' species had shallower rooting depths than less 'tolerant' species. Based on this research and other similar experiments it has been suggested that the ability to develop a shallow root system and avoid the high CO_2 and low O_2 conditions found deeper in the soil is a critical factor in determining the survival of trees on landfills (Gilman *et al* 1982 and 1981; Leone *et al* 1983). It has also been noted that some species with inherent shallower rooting (i.e. even under normal soil conditions) perform better on landfills (Gilman, 1989; Leone *et al* 1983).

The fundamental question is, if for both species the simulated landfill gas resulted in a distinct shallower rooting response that is characteristic of tolerant species how does *Barringtonia* maintain better root function? This question is not restricted to these species or this experiment. Gilman *et al* (1982) showed that Hybrid Poplar (*Populus* spp) and Green Ash (*Fraxinus lanceolata*) both show distinct shallower rooting response to landfill conditions. However their experiment, and the work by Leone and Flower (1982), indicated that Poplar has a greater ability to maintain growth and survival on landfills than Green Ash. It was also observed by Chan *et al* (1991) that the resultant shallower rooting depth in the landfill cover soil also made tree species more susceptible to water stress. Thus it appears that the ability to develop a shallow root system has both advantages and disadvantages especially in dry seasoned climates, and other mechanisms are clearly of use in maintaining root survival and growth under elevated soil CO_2 and low soil O_2 conditions.

The fact that shallower rooting is a common response of plants growing in soils contaminated with landfill gas clearly indicates that most plants try to avoid the resultant

high soil CO_2 and low O_2. However, the ability to maintain a functional root system when landfill gas is unavoidable, like *Barringtonia* in this study, is also clearly beneficial. *Barringtonia* should perform better in soils where there is little atmospheric dilution of landfill gas in surface soil and / or low moisture in the surface soils making shallower rooting of little benefit. The results of this experiment suggest that the key to *Barringtonia*'s ability to maintain root functionality in the unavoidable simulated landfill gas treatment was related to the anatomy of the roots and stem of the species. Unlike *Harpephyllum*, *Barringtonia* had anatomical features which were characteristic of a flood tolerant species. The similarity between flooded and landfill soil atmospheres has commonly lead to the proposal that species adapted to flooding are potentially suitable for planting on landfills (Gilman *et al* 1985; Leone *et al* 1977; Zhang *et al* 1995). It has also been experimentally shown that flooding- tolerant species tend to be more tolerant of landfill gas than flooding- sensitive species (Arthur *et al* 1981). However, little research into the actual anatomical characteristics, which facilitate better performance under landfill gas fumigation, has been conducted.

The most widespread anatomical feature conferring tolerance of flooded soils is an interconnected system of gas spaces (aerenchyma) within the plant (Jackson, 1994). There was clear evidence of lysogenous aerenchyma in the roots of *Barringtonia*, unlike *Harpephyllum* which had very little intercellular root airspace. Aerenchyma is formed either by cell wall separation without collapse, forming a honeycomb appearance of the root cortex (schizogeny) or, as in this case, by programmed cell collapse resulting, normally, in radial air spaces in the cortex (lysogeny) (Jackson & Armstrong, 1999; Laan *et al* 1989). In both *Harpephyllum* and *Barringtonia* there was no apparent effect of the experimental treatments on tissue anatomy and the aerenchyma in the *Barringtonia* roots

was constitutive, which is often a characteristic of flood tolerant species (Drew, 1997). The root aerenchyma in *Barringtonia* would have provided a lower number of energy demanding cortical cells requiring oxygen and formed an internal pathway of high conductivity for gases, thus enhancing internal oxygen diffusion. The ability of aerenchyma tissue to transport oxygen from the shoots to the roots has been shown by experimentally (Jackson & Attwood, 1996; Kludze *et al* 1994; Wiedenroth & Erdmann, 1989). It is also apparent that the mass flow of gas through aerenchyma is unnecessary, as molecular diffusion of oxygen is sufficient to supply root cell respiration, thus making shoot–root oxygen exchange more plausible (Moog & Bruggemann, 1998). Aerenchyma also enhances radial oxygen diffusion allowing gas phase oxygen transport from the central core of the root (Veen, 1987, Wiedenroth, 1993). Efficient radial oxygen diffusion is also important because it also allows for easy movement of oxygen from outside the root through to the central core, as well as easy movement of oxygen within the root. This can increase the availability of the minimal oxygen present within the surrounding soil and within the root. Thus providing the oxygen required for maintenance of nutrient uptake and importantly transportation to the shoots, as illustrated by Topa & Cheeseman, (1994) with their work on *Pinus serotina* under hypoxic growth conditions.

The availability of oxygen to the root cells is also largely dependent on the cell configuration and the degree of secondary thickening. The cortical cells of *Barringtonia* showed a cubic packing arrangement forming concave quadrangulus intercellular air spaces. This cell arrangement was described by Justin and Armstrong, (1987) who , in a study of 91 plant species, identified it as providing maximum gas space per unit tissue volume and the most appropriate cell configuration for plants that rely upon internal ventilation for root aeration. Unlike *Barringtonia*, *Harpephyllum* showed a dense cell

arrangement and very little intercellular airspace. *Harpephyllum* also did not maintain an apparent juvenile root structure like *Barringtonia* but had a high degree of secondary thickening. Secondary thickening rapidly destroys the primary cortex and any primary aerenchyma that may have formed, it also decreases intercellular air space and the potential for internal ventilation (Jackson & Armstrong, 1999; Moog, 1998). Thus it was clear that relative to *Harpephyllum, Barringtonia* had the better root cell configuration to make optimum use of minimal oxygen and allow for maximum internal ventilation.

There were clear differences in stem anatomy between the species, although there was no aerenchyma tissue present in either species, *Barringtonia* had a distinctly more open wood structure and large fibre cells which would be more conducive to internal ventilation. Porosity measurements confirmed that *Barringtonia* stems had a significantly greater amount of airspace than *Harpephyllum* stems. In fact porosity measurements based on Archimedes principle can be up to 60% more accurate than microscopic sections which can overestimate porosity by including all spaces between cells which are not all gas filled (Jackson & Attwood, 1996). High root tissue porosity values are also indicative of the presence of aerenchyma tissue and confirm that intercellular spaces are gas filled (Connel *et al* 1999, Kludze *et al* 1994; Van Noordwijk & Brouwer, 1988). Thus the porosity measurements confirmed the observations and conclusions reached from the root and stem microscopical cross sections. However, they also provided a clear quantitative indication of the difference in anatomy between the two species. *Barringtonia* had mean root and stem porosity values in the simulated landfill gas treatment that were in excess of 9% whilst *Harpephyllum* had significantly lower values that were less than 1.5%. Porosity values less than 7% are found in species that are sensitive to flooded soils (Justin & Armstrong, 1987) and values between 3-5% were associated with very low rates of internal

oxygen diffusion and were responsible for restricted root growth in anaerobic soils (Voesenek *et al* 1999). This provides a clear reason why *Harpephyllum* was unable to maintain root functionality whilst *Barringtonia* could. The lack of internal ventilation within *Harpephyllum* probably resulted in insufficient oxygen availability to the root cells thus reducing nutrient uptake and transport to the shoots. It was apparent that anatomical characteristics associated with internal tissue ventilation were important for better performance under elevated CO_2 and low O_2 conditions and confirmed that characteristics usually associated with flood tolerant species are an important consideration in selecting species for landfills.

In conclusion the results for growth, physiology, and leaf nutrients confirm the hypothesis that the impact of elevated CO_2 and low O_2 is greater on *Harpephyllum* than *Barringtonia*. This reinforced the premise that landfill gas was the key cause for differential performance of these species on the landfill. The results indicated that the key impact of landfill gas was on root system function and the functional relationship between roots and shoots. It was also clear that the roots of both species would prefer to avoid the landfill gas soil conditions, however, this is not always possible or beneficial thus internal tissue ventilation was identified as the key characteristic associated with *Barringtonia* success in an unavoidable landfill gas saturated soil. Elevated CO_2 appears to cause direct toxicity effects on roots which enhances the negative effects of low O_2 on a sensitive species like *Harpephyllum*. However, *Barringtonia* appears to have mechanisms, possibly related to root enzyme activity and aerenchyma tissue, which prevent the negative effects of CO_2 and even make use of CO_2 to reduce the impact of low O_2 on root respiration.

CHAPTER 6: FINAL DISCUSSION

6.1 ENVIRONMENTAL VARIABLES LIMITING VEGETATION GROWTH IN A LANDFILL ENVIRONMENT

In order to improve the stability and aesthetics of operational landfills and increase the scope of rehabilitation planning for closed landfills, successful vegetation establishment is clearly advantageous. Operational sites are permanently undergoing landscape changes in order to accommodate incoming wastes and reduce erosion. Tree damage is not uncommon during construction activity and the value of plants financially and in terms of environmental benefits is not always considered (Yingling *et al* 1979). However, careful planning of site operations and forethought before any revegetation or site construction can easily remedy the impact of earth moving machinery. A more difficult problem to address is the unique and harsh combination of environmental soil variables that challenge plant survival and growth on landfills. The investigations and experiments conducted on the Bisasar Road landfill confirmed the work of others showing that the landfill environments are a formidable challenge to vegetation growth, especially for trees (Chan *et al* 1991; Lan & Wong 1994; Dobson & Moffat 1994; Ettala *et al* 1988; Flower *et al* 1981; Gilman *et al* 1981).

In order to achieve successful revegetation a thorough understanding of the environmental conditions limiting plant growth is essential. The research on the Bisasar Road Landfill highlighted several soil variables that were primarily responsible for poor grass coverage and tree survival and growth. In summary, the results highlighted the importance of soil CO_2 in determining the performance of plants on landfills. However, the compounding effects of other environmental variables such as low soil O_2; changes in soil redox potential; low soil moisture; and high soil conductivity were also identified as potentially

critical variables in determining overall success of a plant. A simple model of the key variables possibly responsible for poor vegetation growth on the Bisasar Road Landfill is provided in Figure 6.1.

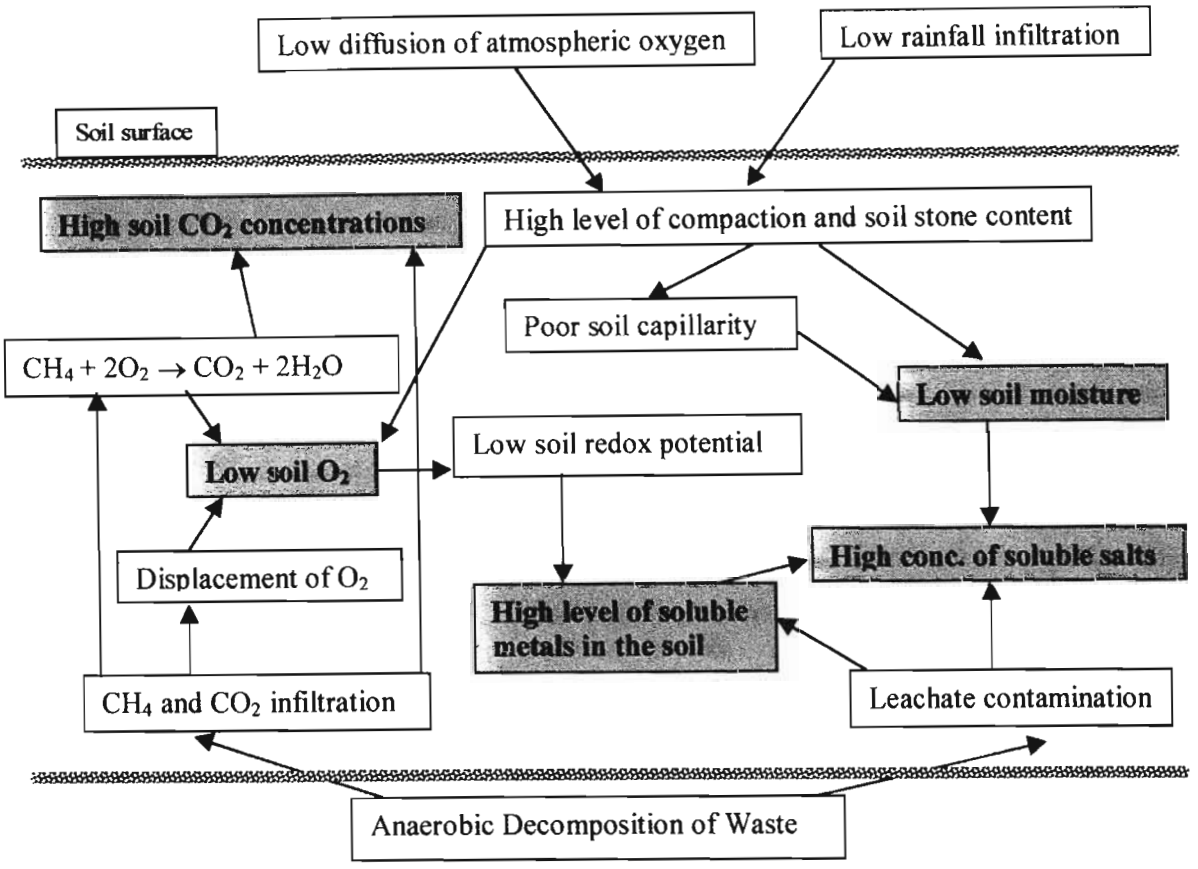


Figure 6.1: A simple model of the inter-relationship of variables that produce the 5 main variables likely to limit plant growth on landfills.

All three studies on the Bisasar Road landfill suggested that landfill gas infiltration into the root zone was the main factor limiting the growth and survival of plants on the landfill. The difference in performance of *Barringtonia racemosa* and *Harpephyllum caffrum* on the landfill (Chapter 4) was similar to that in the simulated landfill gas experiment (Chapter 5), further supporting the premise that landfill gas was the key cause for poor performance of plants on the landfill. The identification of landfill gas as a main factor

limiting plant growth and survival on the Bisasar road landfill is a common conclusion (e.g. Leone & Flower, 1982).

Further, the results of this research provided evidence to support the theory suggested by others that elevated soil CO_2 was the main constituent of landfill gas influencing plant survival and growth on landfills (Chan *et al* 1991, Gilman *et al* 1981, Leone *et al* 1977). A brief summary of the evidence follows. In the first investigation elevated soil carbon dioxide was associated with poor grass colonisation, even though the soil was aerobic (see Chapter 2). The field investigation into tree mortality showed poor tree health was associated with high soil methane and carbon dioxide (Chapter 3). The tree field experiment on the landfill also provided results that suggested that soil CO_2 levels had a key role in influencing plant health (Chapter 4). Further confirmation of the importance of soil CO_2 levels was provided by the fumigation experiment that showed a clear negative effect of elevated CO_2 with or without normal soil O_2 on the physiology and growth of a landfill sensitive species, *Harpephyllum caffrum* (Chapter 5).

However, the experimental evidence suggests that the role of low soil O_2, caused by displacement of soil air by landfill gas and by methane oxidation (Figure 6.1), also needed consideration. Low soil oxygen alone (i.e. without elevated CO_2) can have a negative effect on plant physiology and the root growth of most plants (Huang *et al* 1997, Jackson & Armstrong, 1999). Similar to the findings of Huang *et al* (1997) the fumigation experiment showed that low soil O_2 can make the impact of elevated soil CO_2 more pronounced, especially for CO_2 sensitive species such as *Harpephyllum caffrum.* However, it must be noted that the response of *Barringtonia racemosa* in the fumigation experiment clearly showed that there are possible mechanisms that allow some species to

avoid the negative effects of both low O_2 and elevated soil CO_2. The general performance of *Barringtonia racemosa* was better than that of most other species and the possible mechanisms allowing this will be discussed further below (Section 6.2).

The results of this study provide an opportunity for the evaluation of threshold levels of soil CO_2 and O_2 that are likely to be problematic for plants on landfills. The colonisation of grass appeared to be limited by a root zone CO_2 level of about 14%, even with a relatively aerobic soil of about 12% O_2. However, the soil gas concentrations were not quantified over an extended period of time therefore sporadic or episodic pulses of higher soil CO_2 cannot be discounted as the possible cause for poor grass colonisation. An evaluation of the literature (in Chapter 4) indicated that CO_2 levels of 14% can be associated with poor plant growth and high mortality. Recently, Marchiol *et al* (2000) also found that a simulated landfill gas containing 16% O_2, 8% CO_2 and 3% CH_4 caused a delay in seed germination of a number of plant species. Therefore a CO_2 level of 14% and O_2 of 12% could possibly act similarly and delay or even prevent seed germination. Thus, the CO_2 and O_2 values recorded in the bare areas provided a reasonable explanation for a lack of grass. However, there may also have been additive effects of other adverse environmental conditions, such as soil moisture limitations, albeit these conditions could have been a resultant effect of the lack of grass cover, initially caused by soil gas conditions, but they could then subsequently limit further plant colonisation.

In the field experiment assessing tree performance on the landfill (Chapter 4), the same topsoil was used on the control site and on the landfill. A comparison of the soil variables between the topsoil on the control and that on the landfill during the experiment, provided an indication of the changes the landfill environment can have on soil quality. The topsoil

on the landfill was found to have lower soil moisture and soil O_2, and higher soil CO_2 and extractable Mn in comparison to the topsoil on the control site. Although the change in Mn was considered a possible indicator of soil quality deterioration it was considered unlikely to be problematic for the trees during the experiment and will be discussed later. However, the trees planted on the landfill plot with a topsoil layer still experienced a relatively high level of mortality (24%) during the 435 day experiment. Based on the changes in the topsoil variables, the most likely soil variables responsible for the mortalities were soil moisture, soil O_2 and soil CO_2. The analysis of rooting depth indicated that roots were restricted to a soil depth at which CO_2 levels were less than 20-27% and O_2 levels were greater than 1-2%. Considering it was shown that the CO_2 levels decreased and O_2 levels increased towards the soil surface, the majority of the tree root systems on the experimental landfill plots were probably exposed to less extreme soil gas conditions. In order to consider further the concentration thresholds for soil CO_2 and O_2 and plant response the discussion will focus on *Harpephyllum caffrum*. This species appeared to be sensitive to the landfill environment and its response to elevated soil CO_2 and low soil O_2 were assessed in both the field and fumigation experiment.

In the field experiment *Harpephyllum caffrum* experienced 57% mortality on the landfill topsoil plot within 187 days with the mean CO_2 of 25% and mean O_2 level of 3%. However, in the fumigation experiment, which exposed *Harpephyllum caffrum* roots to similar CO_2 (25%) and soil O_2 (5%) concentrations to the field experiment, a slow deterioration of health but no mortalities during the 140 day experiment was observed. Although mortalities were likely in the long term, the difference in the duration of the two experiments (47 days) was unlikely to completely explain the higher mortality seen in the field experiment. It must be acknowledged that the fumigation experiment was based in a

greenhouse that would have provided optimum growth conditions, and the plants were regularly watered. Therefore the mortality of *Harpephyllum* trees in a relatively shorter time period on the landfill was probably due to the negative additive effects of other environmental stresses found in the field. Figure 6.1 provided a summary of some of the possible below ground variables, however, important above ground variables could include increased stress due to high winds, dust, and possibly air pollution.

One of the key variables that may influence the severity of the effects of soil CO_2 is available soil moisture (Figure 6.1). Low soil moisture was correlated with poor grass colonisation and poor survival of some trees in the field investigations. The application of topsoil over the cover material was found to improve soil moisture levels and also tree survival and growth in the field experiment. Improved soil moisture conditions are usually associated with better soil structure, as was provided by the topsoil layer. However, the quality of cover material used on landfills is usually poor due to availability and high cost of good quality topsoil (Flower, *et al* 1981). High stone content can reduce soil capillarity, thus reducing the upward migration of moisture (Heilmann, 1981; Insley & Carnell, 1982). Soil moisture levels are further limited by the practice of compacting cover soils (Butt *et al* 1999; Flower *et al* 1981; Greacen & Sands, 1980), in order to reduce water infiltration which causes leachate production (Cooper *et al* 1997), and to maximise fill space (Flower, *et al* 1981). Therefore, the low soil moisture levels found on the Bisasar Road Landfill were not unusual and it is not surprising that low soil moisture has been highlighted as a problem for plant growth on landfills (Gendebien, *et al* 1992; Liang *et al* 1999). It is important to note that low soil moisture problems on landfills are particularly problematic in areas that receive relatively low and seasonal rainfall such as in the Bisasar Road Landfill in Durban and in most of southern Africa (Chapter 1, Table 2.1).

Low soil moisture conditions can also compound the effects of other variables such as the high concentrations of soluble salts in the soil. The soil conductivity levels in the landfill cover material were in excess of the minimum standards for woodland establishment (Moffat & Bending, 1992) and in conjunction with low moisture availability the potential for osmotic and ionic stress on the vegetation becomes more severe (Bradshaw & Chadwick, 1980). Although this is a potential problem the investigation of grass growth indicated that the natural colonisers of the site were generally tolerant of high soil conductivity. Therefore, it was not the key reason for patchy grass growth. However, the trees generally responded well to the topsoil layer, which had a significantly lower soil conductivity and higher soil moisture levels, suggesting that on the poor landfill cover soils the level of soluble salts in the soil may have had an impact on tree growth and survival. Thus the relatively high conductivity of the soil in conjunction with the low soil moisture conditions were likely variables responsible for enhancing the severity of the effects of high soil CO_2.

High concentrations of soluble salts in landfill soils are generally caused by leachate contamination (Dobson & Moffat, 1994; Lan & Wong, 1994; Menser *et al* 1983; Wong *et al* 1992). The relatively high levels of soil Ca found in the grass field investigation and the field tree experiment can be indicative of leachate contamination of landfill cover material (Hernandez *et al* 1999). Further evidence, such as relatively high soil pH, and high K concentrations found, also suggested that the cover material on the landfill maybe contaminated with leachate (Winant *et al* 1981).

Heavy metal (Tong & Wong, 1984) and possibly chloride contamination of the soil, due to leachate, may result in phytotoxity (Menser *et al* 1983; Ettala, 1988). However, analysis of

the total metal content of the landfill cover material and the additional topsoil layer during the field experiment showed that levels of metal contamination of the soil was minimal and unlikely to be phytotoxic. The concentration of heavy metals in leachate from landfills is generally low and does not usually constitute a significant pollution problem (Christensen *et al* 2001). Therefore, metal toxicity was an unlikely reason for poor plant growth and survival on the landfill. Leachate, with high concentrations of ions, can cause changes in the soil chemistry, sometimes resulting in the leaching of soil nutrients (Dobson & Moffat, 1994). This may provide an explanation for the low Mg concentrations measured in the landfill cover material (Chapter 4). It was apparent that leachate contamination of the landfill cover material can result in deterioration of soil quality. However, the evidence from the grass bioassay and the analysis of soil nutrient indicated that soil leachate contamination and the resultant change in soil nutrient content was minimal. Therefore, the influence of leachate was unlikely to have any great effect on plant growth and survival in this research.

An increase in extractable Mn seen in the topsoil placed on the landfill originally raised concerns about leachate contamination. However the lack of significant difference in total Mn concentrations between the experimental plots proved that Mn levels were not due to an external source of contamination. The increasing levels of Mn in the topsoil on the landfill was attributed to the low oxygen levels in the topsoil layer created strong reducing conditions which cause insoluble Mn^{4+} to form the highly soluble Mn^{2+}. This could possibly change the ratio of total manganese to ammonium bicarbonate EDTA extractable manganese (Crawford, 1989; Menser *et al* 1979; Munshower, 1994; Rees, 1982). Mn toxicity in plants is not uncommon, especially under strongly reducing conditions (Gonzalez & Lynch, 1999; Mgema & Clarke, 1995). Mn is usually found to accumulate in

the leaves resulting in a decline in photosynthetic activity by interfering with the activities of the CO_2 reduction cycle (Kitao *et al* 1997). Although net photosynthesis or leaf Mn levels were not measured in the field experiment, they were measured in the simulated landfill soil atmosphere experiment. Although simulated landfill conditions showed a reduction in the net photosynthesis for the landfill sensitive species (*Harpephyllum caffrum*) there was no evidence of elevated leaf Mn levels. It may be inferred from this that Mn toxicity was unlikely to be the cause of poor plant performance on the landfill. However, this is not conclusive and further field measurements that include soil redox potentials and leaf Mn levels would help confirm any detrimental effects of soil Mn.

It can be concluded that landfill gas infiltration into the root zone and the resultant elevated soil CO_2 conditions is the primary cause of poor plant growth on landfills. However, the severity of the effect is largely dependant on species tolerance and the compounding effects of other variables such as low soil oxygen, low soil moisture and possibly leachate contamination of the soil.

6.2 SPECIES TOLERANCE

In the natural environment low soil O_2 conditions are not uncommon. Nearly 6% of the Earth's surface is classified as wetland and is flooded for at least part of the year (Maltby, 1991), resulting in low soil oxygen conditions (Crawford, 1989). Therefore plants tolerant to low soil oxygen conditions, similar to that found on landfills, are not uncommon. *Barringtonia racemosa, Hibiscus tiliaceus* and *Combretum erythrophyllum,* which performed best on the landfill in this investigation, grow in natural habitats bordering swamps and river courses. Interestingly, the tree species that performed poorly on the landfill were associated with natural habitats that were unlikely to have waterlogged soils.

These species included *Erythrina lysistemon, Rhus lancea, Acacia sieberiana, Strelitzia nicolai,* and *Harpephyllum caffrum* (Palgrave, 1984; Pooley, 1994). This relationship between species from waterlogged habitats and tolerance to landfill conditions has been reported by a number of investigators (Arthur *et al* 1981; Chan *et al* 1991; Crook, 1992; Gilman *et al* 1985; Leone *et al* 1977). This apparent relationship is obviously unlikely to be associated with soil moisture similarities between the two habitats, as the landfill soil moisture levels can be relatively low (Crook, 1992; Gilman *et al* 1985). The relationship is attributed to the similarity in soil O_2 levels between waterlogged habitats and landfill cover soils (Arthur *et al* 1981; Gilman *et al* 1985).

However, it is important to note that not all waterlogged soils have low soil O_2. Turbulent flood waters often have sufficient oxygen in the water for aerobic respiration of roots (Gill,1970; Mckersie & Leshem, 1994). Therefore, one has to be more specific about the characteristics of the waterlogged habitat. Those characterised by more permanent and stagnant water, such as swamps and marshes, tend to have much lower soil oxygen levels (Mckersie & Lesham, 1994) and, therefore, more likely to be habitats with species tolerant of low soil O_2. It is also critical to consider the potential difference in soil moisture content between a waterlogged soil and a dry landfill cover soil. Low soil moisture conditions and low oxygen conditions seldom occur together in natural habitats. Species that inhabit areas with soils saturated with stagnant water during the wet season, but are also exposed to low soil moisture conditions in the dry season, maybe tolerant of low soil O_2 and low soil moisture conditions. This is the case for *Barringtonia racemosa* which has a natural distribution within swamp forest associated with rivers, estuaries or coastal areas, but grows well in wet and dry conditions (Palgrave, 1984; Pooley, 1994). It is rather unusual for a species to be tolerant of such a broad range of soil moisture conditions, however it is

probably one of the key reasons contributing to the good performance of *Barringtonia racemosa* on the landfill.

However, the natural habitat of a species is not always a clear guideline to the potential performance of a species in landfill environments. For example *Syzygium cordatum* is usually found on river banks (Palgrave, 1984; Pooley, 1994). Therefore, it would probably be exposed to waterlogged soils or at least to periods of waterlogging, however, this species was one of the most sensitive species to the landfill conditions. The results of the tree investigation (Chapter 3) indicated that the poor performance of *Syzygium cordatum* on the landfill was related to low soil moisture, which it may not experience in its natural habitat. It could also be attributed to river flood waters, as opposed to stagnant water, being relatively rich in O_2 therefore levels of soil O_2 may not be as low as landfill soils. Although these ideas are all speculative, it highlighted the difficulties in trying to correlate the similarities between a species natural habitat description and a landfill environment. It is apparent from this investigation and that by Arthur *et al* (1981) that it can sometimes provide an indication of species potential, however, the similarity between a waterlogged soil and a landfill cover soil is only apparent on a very simplistic level i.e. potentially low soil O_2. In waterlogged soils the prime cause of poor plant performance is the poor availability of O_2 for the roots (Jackson & Armstrong, 1999). However, this investigation and other landfill research indicate that the prime cause of poor performance of plants on landfills is elevated soil CO_2, to which low O_2 has an additive effect (Chan *et al* 1991; Gilman *et al* 1981, Leone *et al* 1977). Therefore the primary determinant of plant health and performance differs between waterlogged soils and landfill cover soils. It is also important to consider the difference in soil moisture between the two habitats.

It was apparent in both the field and fumigation experiment that tree roots of all species investigated tried to avoid high levels of soil CO_2 and low O_2 through shallower rooting. However, the severity of this response was more marked in those species that performed poorly on the landfill and the response appeared to be mainly driven by elevated soil CO_2 and not low O_2. The conclusion that soil CO_2 was the driving force behind shallower rooting depths on landfills has been reached by others (Chan *et al* 1991; Gilman *et al* 1981). However shallower rooting has previously been considered a response that is beneficial for survival on landfills, as it allows for the avoidance of adverse soil atmosphere conditions found deeper within the soil (Gilman *et al* 1982; Gilman *et al* 1981; Leone *et al* 1983). It is clear that shallower rooting can allow for avoidance of poor soil atmosphere conditions, however, as suggested by Chan *et al* (1991), it results in a greater susceptibility of plants to water stress, especially in arid climates and where there are low soil moisture levels. Therefore species performance on landfills, as indicated by the root morphology results of this study, is more likely to be associated with ability of species to maintain relatively deeper rooting despite the poor soil atmosphere conditions.

The ability to maintain a functional root system in the presence of elevated soil CO_2 is critical to achieving greater rooting depth. The results of the fumigation experiment indicated that for *Barringtonia racemosa*, this ability is closely related to an inherent specialised tissue arrangement of the roots and shoots, increasing intercellular airspace. This species also appears to maintain health under poor soil atmosphere conditions through the control of the resource demands of shoot and root through an unknown mechanism involving the avoidance of CO_2 toxicity, which may involve the transport and leaf utilisation of soil CO_2 to it own metabolic advantage.

In comparing the characteristics of *Barringtonia racemosa*, a species that performed well on the landfill, with that of *Harpephyllum caffrum*, a species that performed poorly, some of the possible mechanisms that allowed better species performance have been elucidated. However, the potentially beneficial characteristics, such as relatively high levels of intercellular airspace, need to be investigated in other species in relation to high CO_2 and low O_2 and known landfill performance. If there is a general association between such species characteristics and landfill performance this will considerably facilitate plant species selection for landfill revegetation. This is discussed further in the next section.

6.3 FUTURE LANDFILL MANAGEMENT AND RESEARCH IMPLICATIONS

Successful revegetation of contaminated or difficult sites is often through the use of plant species that have known tolerances to the problematic environmental factors, especially when used in conjunction with ameliorative procedures that are focused on these environmental factors (Bradshaw, 1984).

Other than this study, little research in South Africa has been done on the tolerance of indigenous species to landfill environmental conditions. The screening of indigenous species suitable for landfill revegetation, by field experiment, has been carried out in Europe and America, however the task is somewhat daunting on the African continent. For example KwaZulu-Natal alone has over 750 indigenous tree species, which is over ten times as many tree species as are native to the whole of Europe (Pooley, 1994). The biodiversity is high and our ecological knowledge about individual tree species is very limited. Thus, it is certainly not possible to investigate all of the species through field trials of relatively long duration and the random selection of tree species has high costs relative

to success or benefits. Therefore, the knowledge we have about the environmental variables that are a problematic for plant growth and the characteristics of species that have performed well on landfills need to be used to increase the efficiency and success of species selection for landfill revegetation. Although this study provides information about the suitability of 10 indigenous species for landfill revegetation, it is the knowledge about the characteristics of these species and the key landfill conditions that determine species success, which are the tools that will be useful for landfill practitioners. They can assist in further species selection and amelioration of landfill conditions to ensure greater success of landfill revegetation.

Landfill gas infiltration into the root zone has been identified as a key variable responsible for poor plant survival and growth. More specifically species that can grow under elevated soil CO_2 conditions need to be identified. Especially those that can tolerate the enhanced negative effects created by low soil O_2, low soil moisture, and high soil conductivity. Although all species appear to prefer to avoid the elevated soil CO_2 and low O_2 conditions through shallower rooting, the selection of species based on their ability to maintain a functional root system when avoidance is not beneficial is likely to yield useful species. This ability may be related to inherent high levels of root and stem porosity and the presence of root aerenchyma tissue, therefore these characteristics could be used as initial selection criteria. It is also apparent that the natural habitat of a species can provide an indication of its potential performance on a landfill. This study indicates that the screening of species that naturally occur in waterlogged habitats will yield a number of useful species for landfill revegetation.

Applying knowledge about the limiting soil variables and other factors causing plant death is important in site preparation for revegetation. The application of topsoil over the normal landfill cover material significantly improved the health and survival of most species in this study. This appeared to be mainly due to the reduced additional effect of high soil conductivity and low soil moisture on the impact of elevated soil CO_2. However, ameliorating high soil CO_2 levels is more problematic. Procedures to reduce landfill gas infiltration into the root zone of plants have been suggested by a number of researchers. These mainly involve barriers or liners below the topsoil layer that divert landfill gas away from the root zone of plants (Gilman *et al* 1985; Spreull & Cullum 1987). In operational sites these measures work similarly to the final landfill cap in separating the surface soils from the underlying waste. This may be a useful technique, however capping material is expensive and for large areas that are only temporarily closed for several years the expense may be restrictive. Therefore, amelioration of the soil CO_2 levels is limited and selection of species tolerant to these conditions appears to be, if possible, the most appropriate solution.

The mechanism by which the concentrations of Mn in the soil increased by six fold in the topsoil placed over the landfill cover material, in this study, needs further research. It has been concluded that the increase is not due to soil contamination from the underlying waste. The results suggest it may be due to changes in soil redox potential due to the low O_2 conditions. However, the relationship between extractable Mn and soil redox potential needs to be researched and the possible impact on plants needs to be determined.

The importance of CO_2 raises questions about the role of methane oxidation in the surface soils and the success of vegetation establishment. Methane oxidation utilises available soil O_2 and results in conversion of relatively harmless methane into CO_2. The global

contribution of methane from landfills has caused concern about the 'greenhouse' effect (Diot *et al* 2000). There is an ever-increasing interest in methane oxidation in landfill cover soils as a natural treatment method for reducing methane emissions into the atmosphere (Visvanathan *et al* 1999). Methane is reported to be 20 times more effective at trapping heat in the atmosphere than carbon dioxide (Haarstad, 1997). Therefore, in order to reduce the 'greenhouse' effect, it may be said that there is a social demand for higher rates of methane oxidation into carbon dioxide, by bacteria in landfill cover materials (Borjesson & Svensson, 1997; De Rome *et al* 1997; Visvanathan *et al* 1999). This demand has resulted in a surge of research into methods of enhancing methane oxidation in landfill cover soils (Boeckx & Van Cleemput 1996; De Visscher *et al* 1999; Willison *et al* 1996). However, methane oxidation increases the levels of carbon dioxide and reduces the levels of oxygen in the soil (De Rome *et al* 1997; Dobson & Moffat, 1994; Haarstad, 1997; Hoeks, 1983). This could make revegetation and stabilisation of landfill sites more difficult. Therefore there is a need for research into the possible implications that enhancing methane oxidation in cover soils may have on revegetation success, as it is clear that the objectives can be in conflict

Research into landfill revegetation allows for a greater understanding of the inter-relationships between the environmental variables resulting in poor vegetation growth, and the mechanisms of species tolerance to these conditions. With this knowledge, management guidelines for the revegetation of operational and complete landfills can be designed, which can help ensure long term successful site rehabilitation, and thus site closure permiting and sustainable land use for future generations.

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